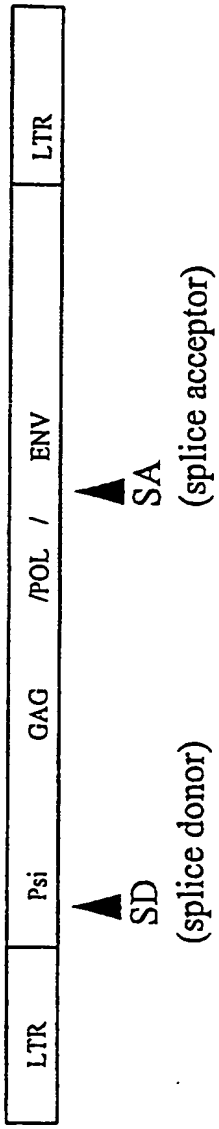
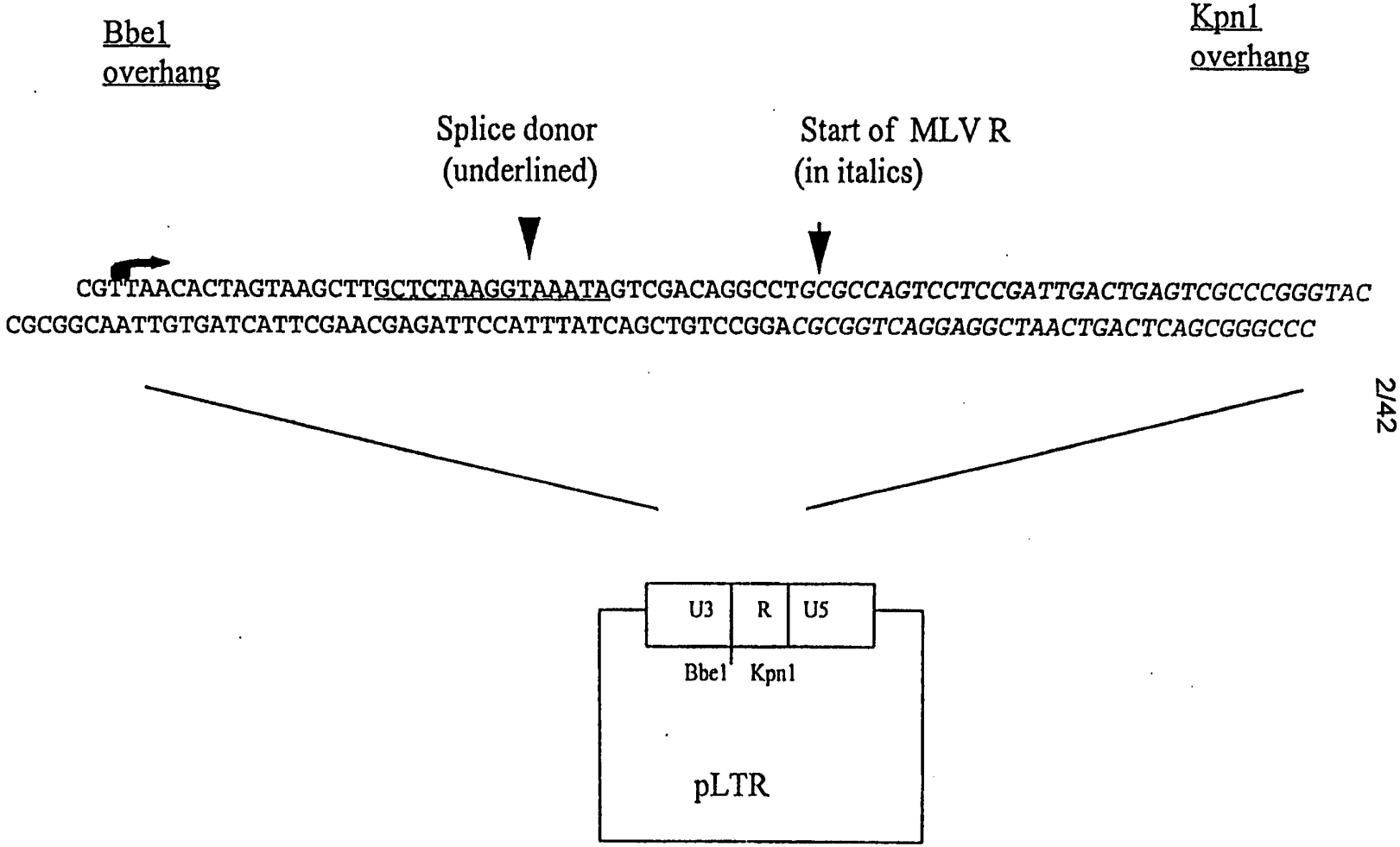


Figure 1

Figure 2



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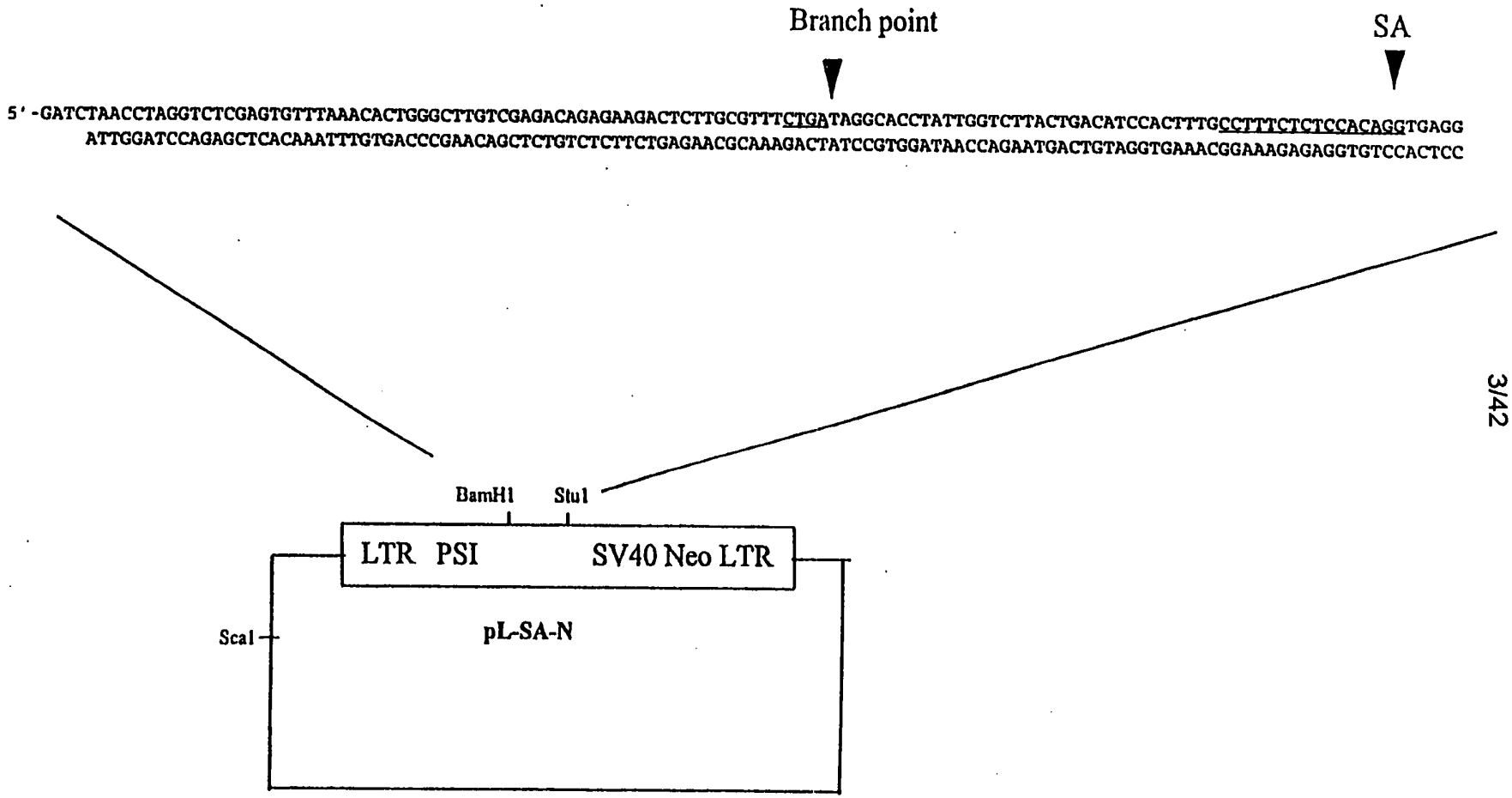
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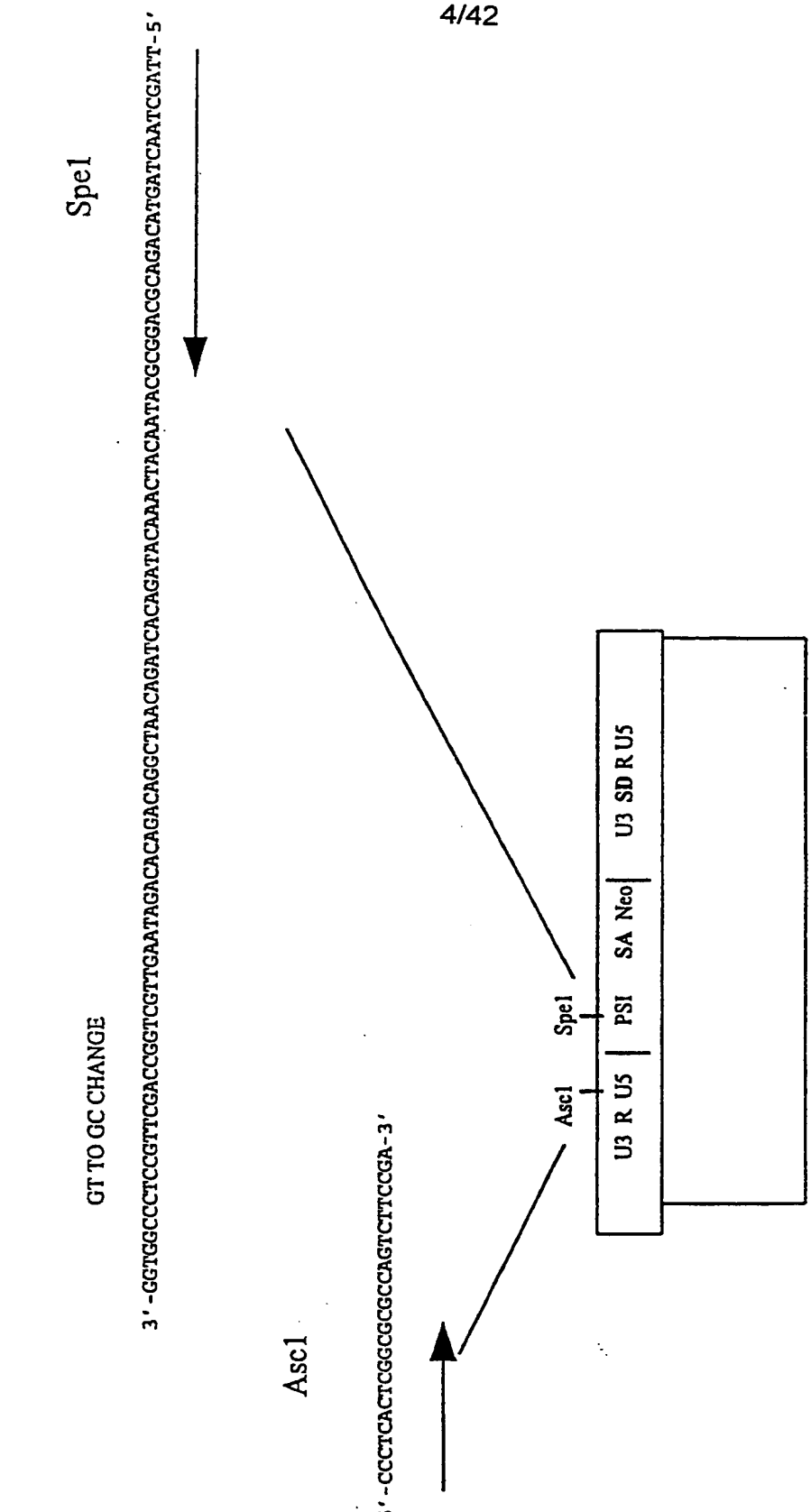
Figure 4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Spe1
Asc1
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PSI
SA Neo
U3 SD R U5

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Figure 5

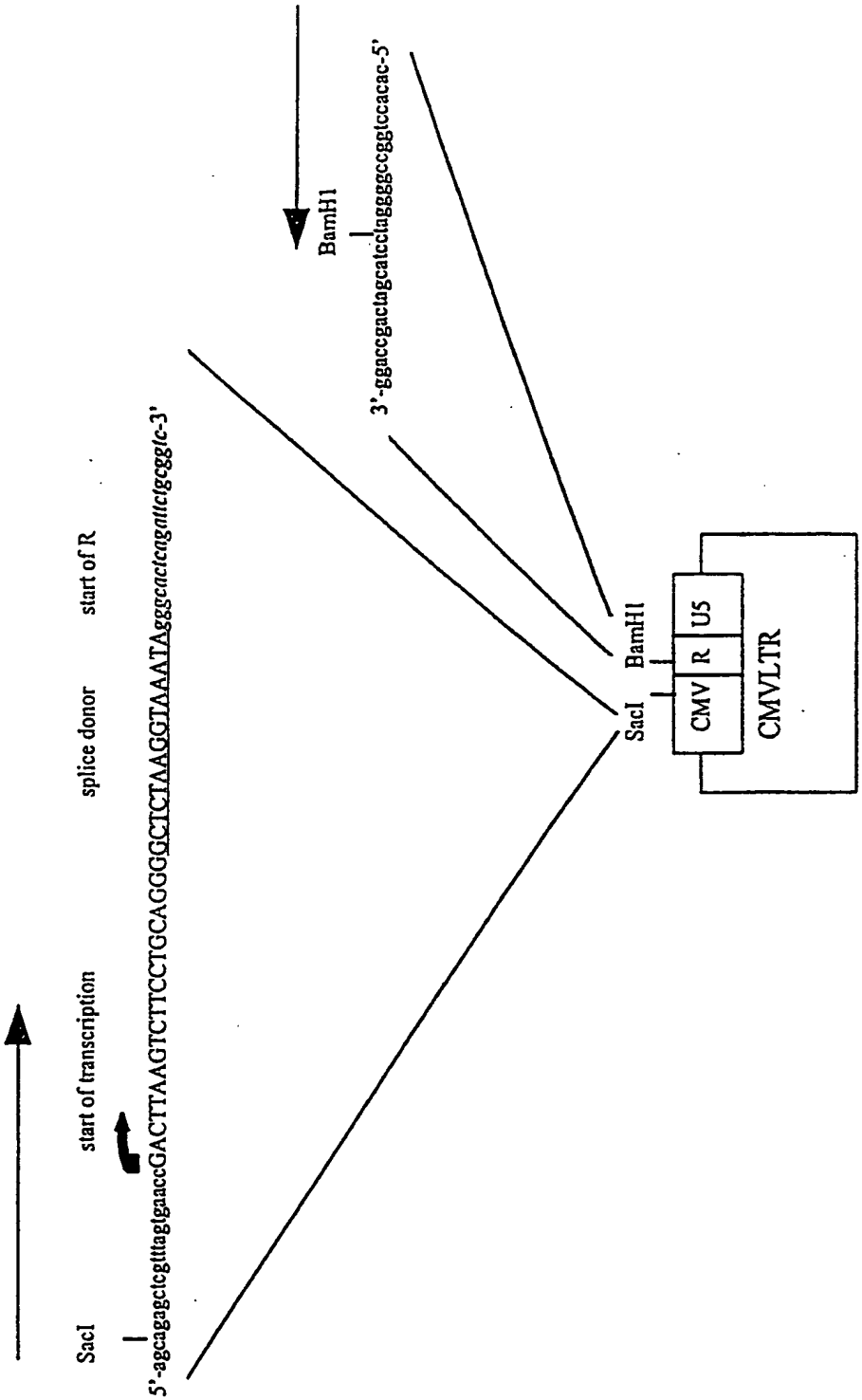
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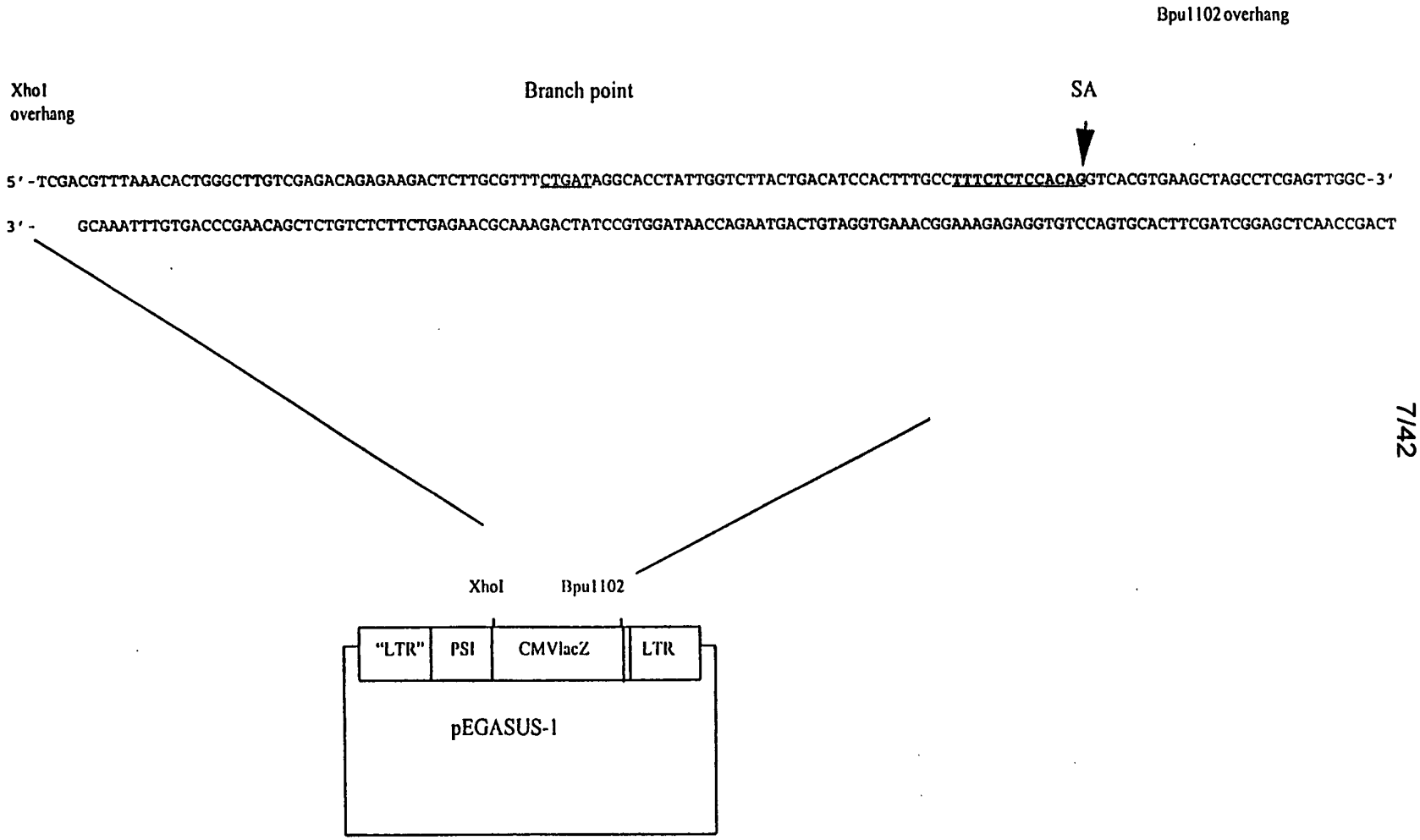
Figure 6

Figure 7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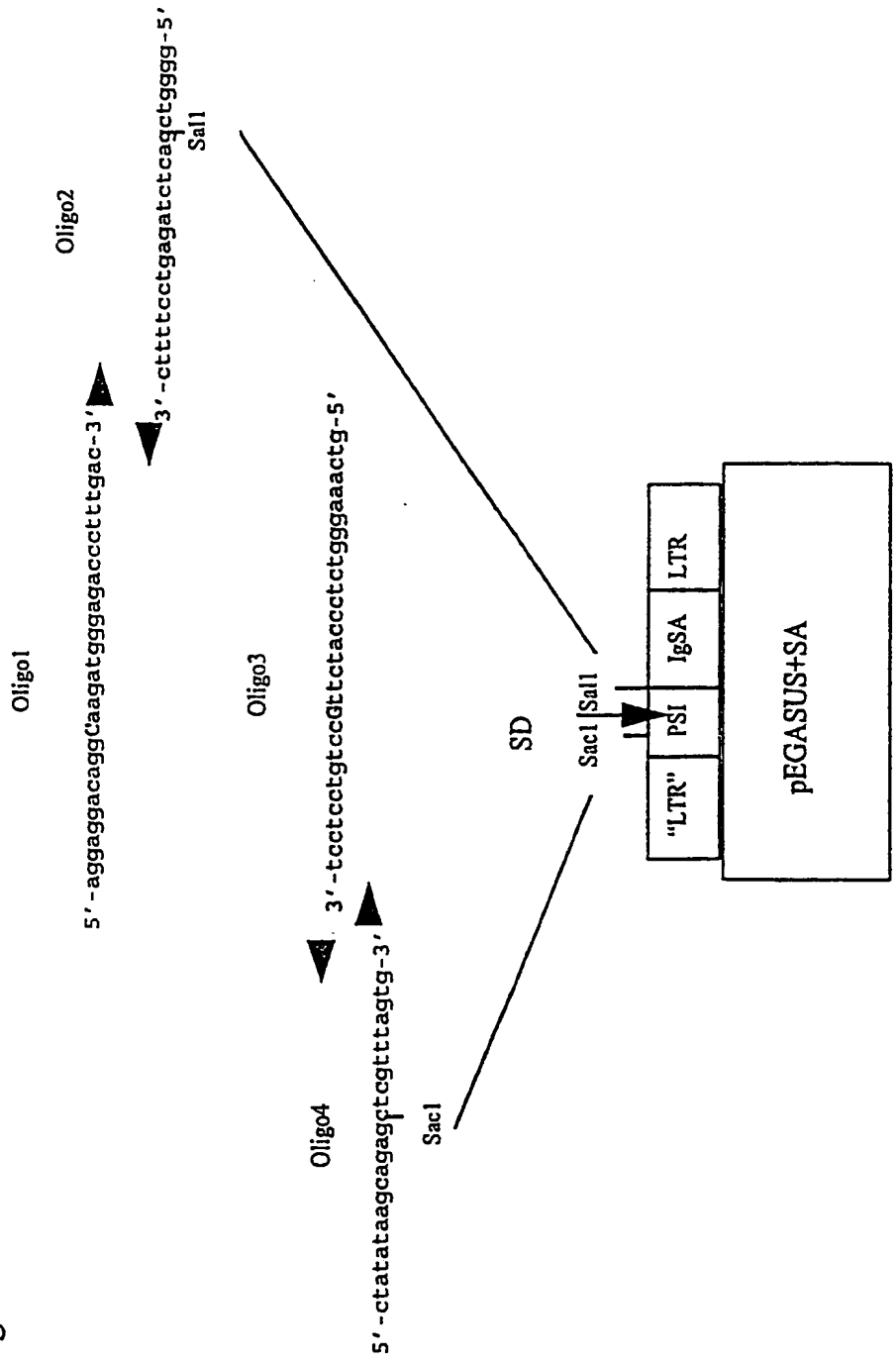
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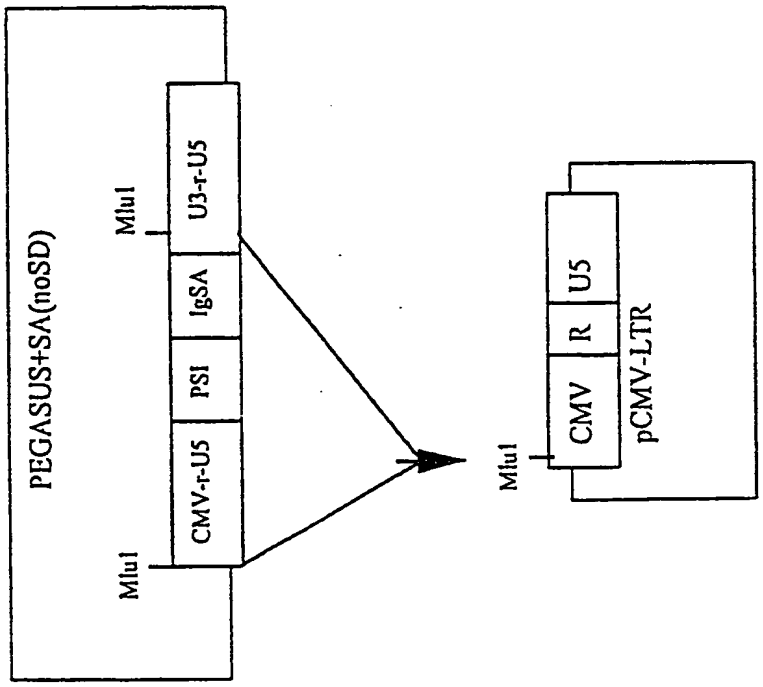
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Figure 9

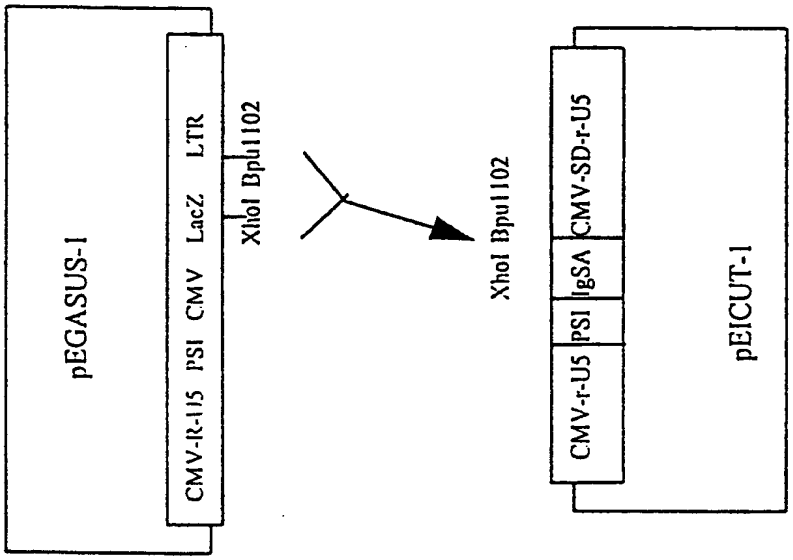

Figure 10

Figure 11

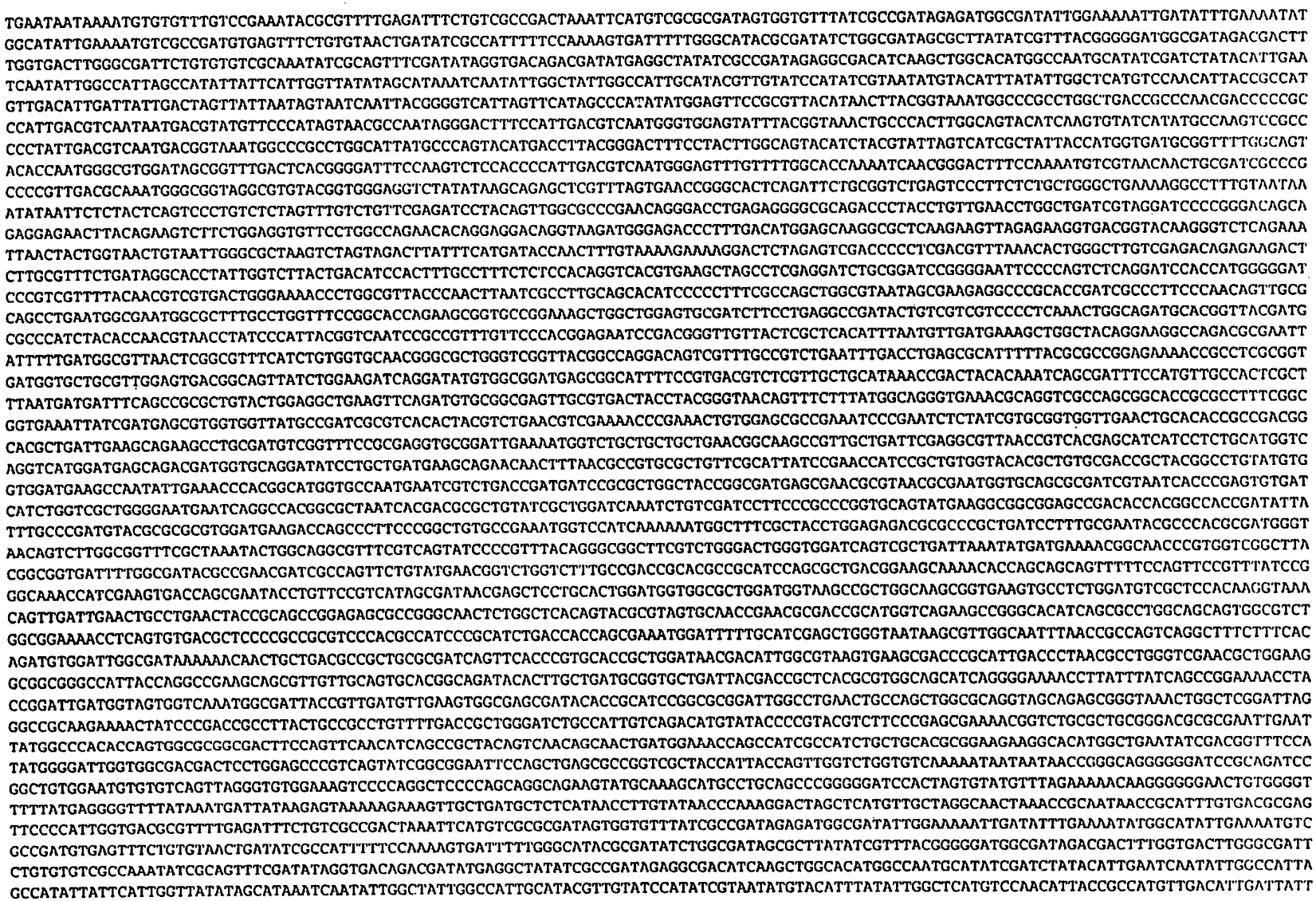
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Figure 11 continued

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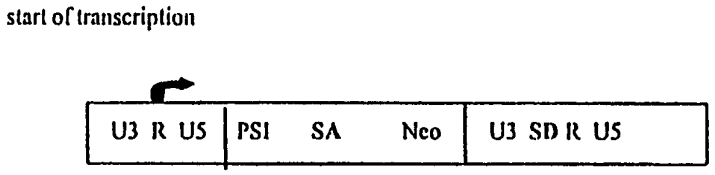
CGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGC
GATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTT
GGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGACC
AAGCGACGCCCAACCTGCCATCACGATGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGATAGCGATAAGGATCGATCCGGGCTGATCCTCAGTACTAC
AATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCAT
GTGTCAGAGGTTTTCACCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAA
TGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCG
TGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAA
CAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCAT
ACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACT
TCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCAC
GATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGC
CCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCA
GGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAA
AAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCG
CGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCT
TCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTC
AAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCAC
GCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGA
CTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGGCTCGACAGATCT

Figure 12

(A) pICUT vector in transfected cells



(B) pICUT vector in transduced cells

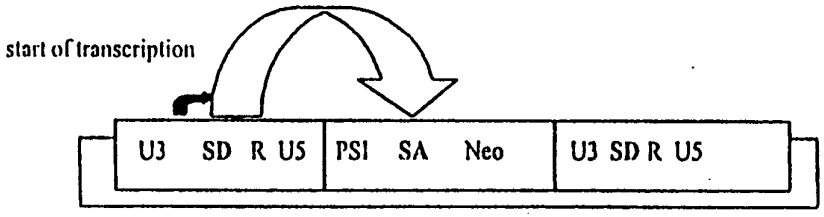
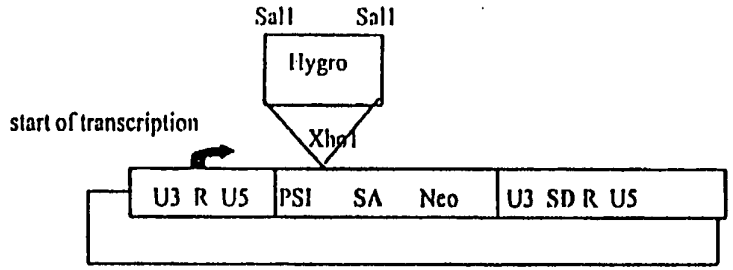


Figure 13

(a) Vector configuration in transfected cells



(B) Vector configuration in transduced cells

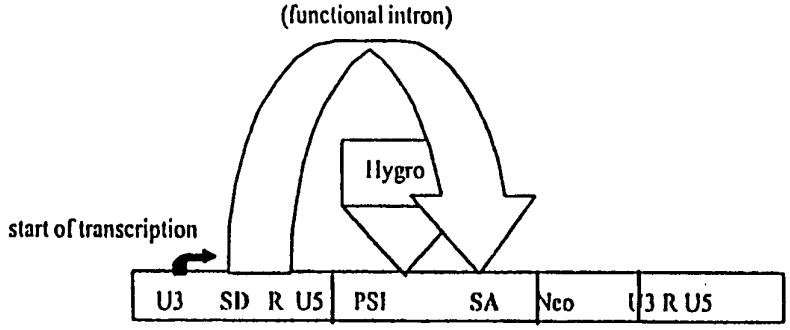


Figure 14

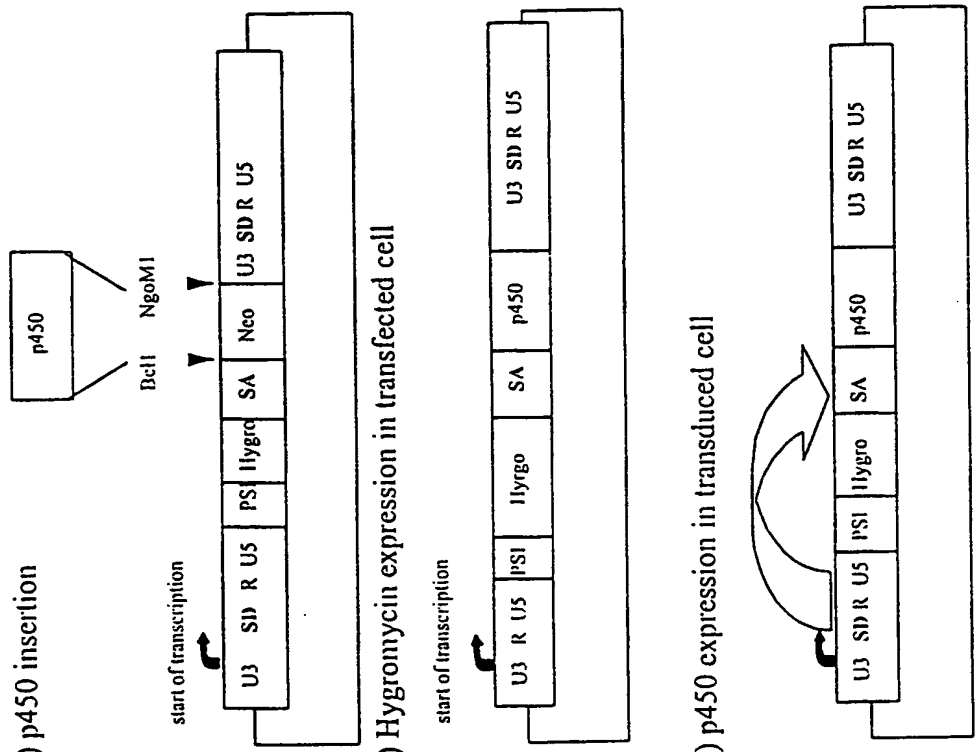


Figure 15

```
3'end of pol      5'-ATG CGT TCA ACG CTC TCA AAA CCC CTT AAA AAT AAG
5'altered 4070A   5'-ATG GCC AGA AGC ACC CTG AGC AAG CCA CCC CAG GAC

                      GTT AAC CCG CGA GGC CCC CTA ATC CCC-3'
                      AAA AAT CCC TGG AAA CCT CTG ATC GTC-3'
```

Figure 16

ATGGCCAGAA GCACCCTGAG CAAGCCACCC CAGGACAAAA TCAATCCCTG GAAACCTCTG
ATCGTCATGG GAGTCCTGTT AGGAGTAGGG ATGGCAGAGA GCCCCCATC AGGTC
TTTAATGTAA CCTGGAGAGT CACCAACCTG
ATGACTGGGC GTACCGCCAA TGCCACCTCC CTCCTGGGAA CTGTACAAGA TGCCTTCCCA
AAATTATATT TTGATCTATG TGATCTGGTC GGAGAGGAGT GGGACCCTTC AGACCAGGAA
CCGTATGTCG GGTATGGCTG CAAGTACCCC GCAGGGAGAC AGCGGACCCG GACTTTTGAC
TTTTACGTGT GCCCTGGGCA TACCGTAAAG TCGGGGTGTG GGGGACCAGG AGAGGGCTAC
TGTGGTAAAT GGGGGTGTGA AACCACCGGA CAGGCTTACT GGAAGCCCAC ATCATCGTGG
GACCTAATCT CCCTTAAGCG CGGTAACACC CCCTGGGACA CGGGATGCTC TAAAGTTGCC
TGTGGCCCCT GCTACGACCT CTCCAAAGTA TCCAATTCCT TCCAAGGGGC TACTCGAGGG
GGCAGATGCA ACCCTCTAGT CCTAGAATTC ACTGATGCAG GAAAAAAGGC TAACTGGGAC
GGGCCCAAAT CGTGGGGACT GAGACTGTAC CGGACAGGAA CAGATCCTAT TACCATGTTC
TCCCTGACCC GGCAGGTCCT TAATGTGGGA CCCCGAGTCC CCATAGGGCC CAACCCAGTA
TTACCCGACC AAAGACTCCC TTCCTCACCA ATAGAGATTG TACCGGCTCC ACAGCCACCT
AGCCCCCTCA ATACCAGTTA CCCCCCTTCC ACTACCAGTA CACCCTCAAC CTCCCCTACA
AGTCCAAGTG TCCCACAGCC ACCCCCAGGA ACTGGAGATA GACTACTAGC TCTAGTCAAA
GGAGCCTATC AGGCGCTTAA CCTCACCAAT CCCGACAAGA CCCAAGAATG TTGGCTGTGC
TTAGTGTCGG GACCTCCTTA TTACGAAGGA GTAGCGGTCG TGGGCACTTA TACCAATCAT
TCCACCGCTC CGGCCAACTG TACGGCCACT TCCCAACATA AGCTTACCCT ATCTGAAGTG
ACAGGACAGG GCCTATGCAT GGGGGCAGTA CCTAAAACTC ACCAGGCCTT ATGTAACACC
ACCCAAAGCG CCGGCTCAGG ATCCTACTAC CTTGCAGCAC CCGCCGGAAC AATGTGGGCT
TGCAGCACTG GATTGACTCC CTGCTTGTCC ACCACGGTGC TCAATCTAAC CACAGATTAT
TGTGTATTAG TTGAACTCTG GCCCAGAGTA ATTTACCACT CCCCCGATTA TATGTATGGT
CAGCTTGAAC AGCGTACCAA ATATAAAAGA GAGCCAGTAT CATTGACCCT GGCCCTTCTA
CTAGGAGGAT TAACCATGGG AGGGATTGCA GCTGGAATAG GGACGGGGAC CACTGCCTTA
ATTAAAACCC AGCAGTTTGA GCAGCTTCAT GCCGCTATCC AGACAGACCT CAACGAAGTC
GAAAAGTCAA TTACCAACCT AGAAAAGTCA CTGACCTCGT TGTCTGAAGT AGTCCTACAG
AACCGCAGAG GCCTAGATTT GCTATTCCTA AAGGAGGGAG GTCTCTGCGC AGCCCTAAAA
GAAGAATGTT GTTTTTATGC AGACCACACG GGGCTAGTGA GAGACAGCAT GGCCAAATTA
AGAGAAAGGC TTAATCAGAG ACAAAAACTA TTTGAGACAG GCCAAGGATG GTTCGAAGGG
CTGTTTAATA GATCCCCCTG GTTTACCACC TTAATCTCCA CCATCATGGG ACCTCTAATA
GTACTCTTAC TGATCTTACT CTTTGGACCT TGCATTCTCA ATCGATTGGT CCAATTTGTT
AAAGACAGGA TCTCAGTGGT CCAGGCTCTG GTTTTGACTC AGCAATATC CCAGCTAAAA
CCCATAGAGT ACGAGCCATG A

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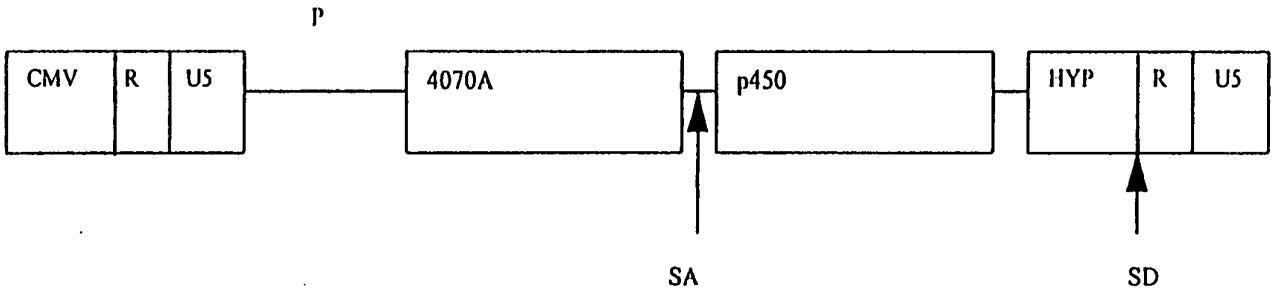
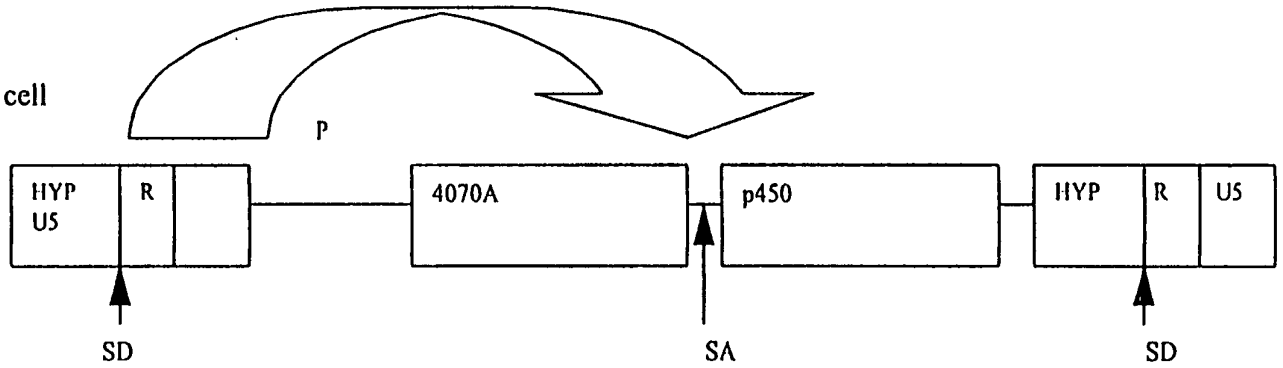
Figure 17

Packaging cell



Transduced cell



CMV= CMV Promoter
HYP= Hypoxia responsive promoter
P= MLV packaging signal

4070A= MLV amphotrophic Env gene
p450= p450 reducatase encoding cDNA
SD= Splice donor
SA= Splice acceptor

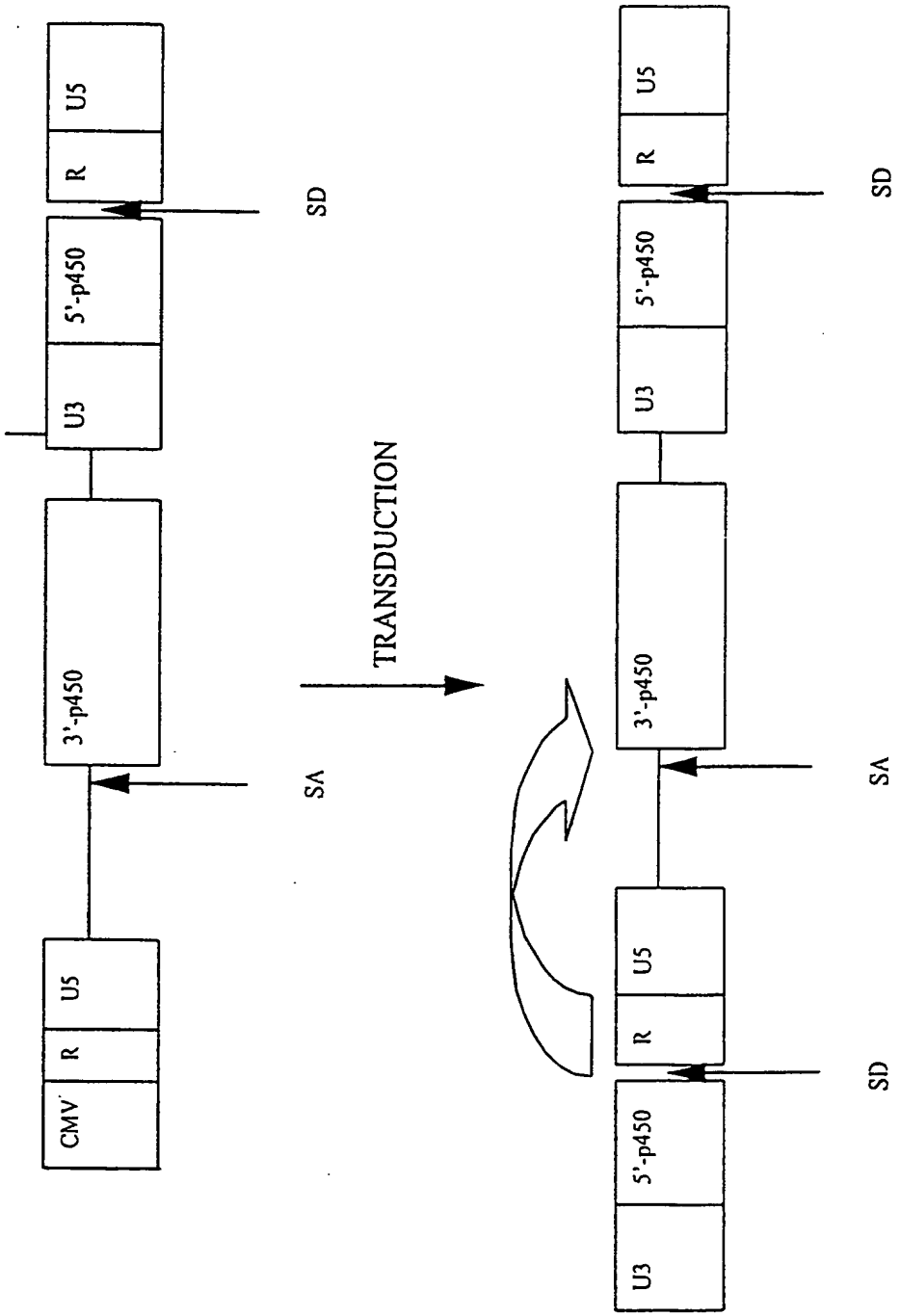
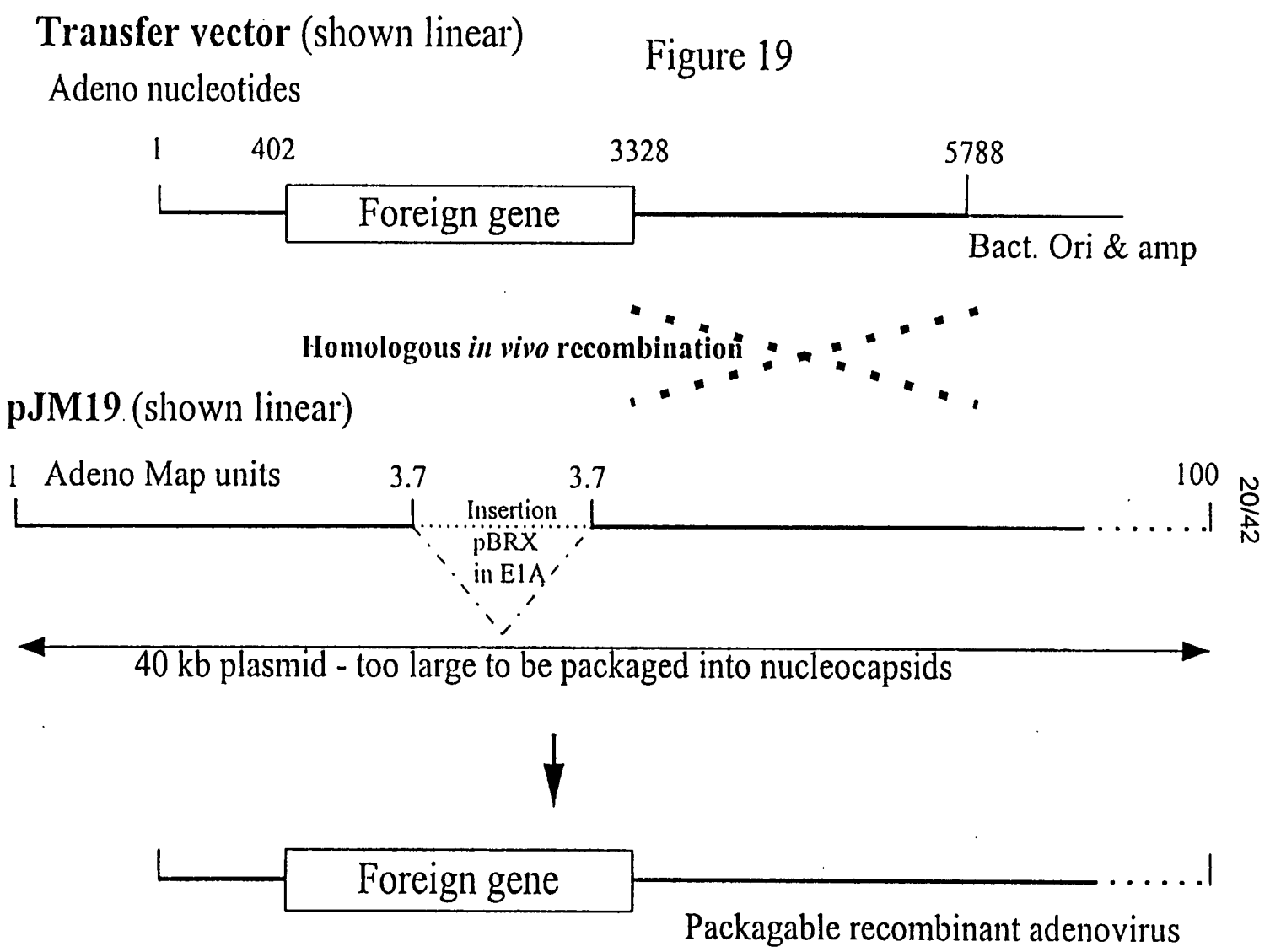

CMV
R
U5
3'-p450
SA
NheI
U3
5'-p450
R
U5
SD
TRANSDUCTION
U3
5'-p450
R
U5
SD
3'-p450
SA
U3
5'-p450
R
U5
SD

Figure 18

Figure 19


Transfer vector (shown linear)
Adeno nucleotides
1
402
3328
5788
Foreign gene
Bact. Ori & amp
Homologous in vivo recombination
pJM19 (shown linear)
1 Adeno Map units
3.7
3.7
100
Insertion
pBRX
in E1A
40 kb plasmid - too large to be packaged into nucleocapsids
Foreign gene
Packagable recombinant adenovirus

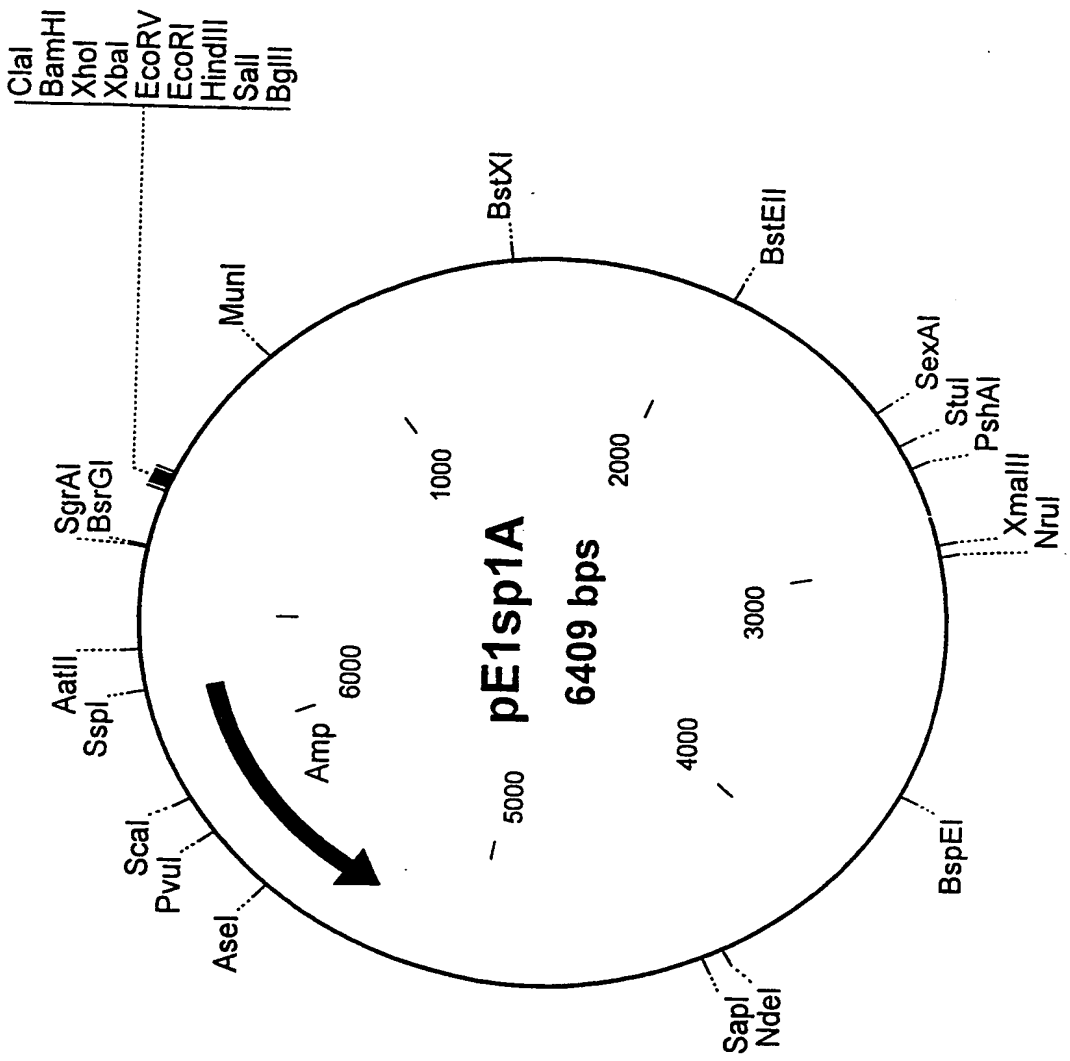

ClaI
BamHI
XhoI
XbaI
EcoRV
EcoRI
HindIII
SalI
BglII
MunI
BstXI
BstEII
SexAI
StuI
PshAI
XmaIII
NruI
BspEI
SapI
NdeI
AseI
PvuI
ScaI
SspI
AatII
SgrAI
BsrGI
Amp
pE1sp1A
6409 bps
1000
2000
3000
4000
5000
6000

Figure 20

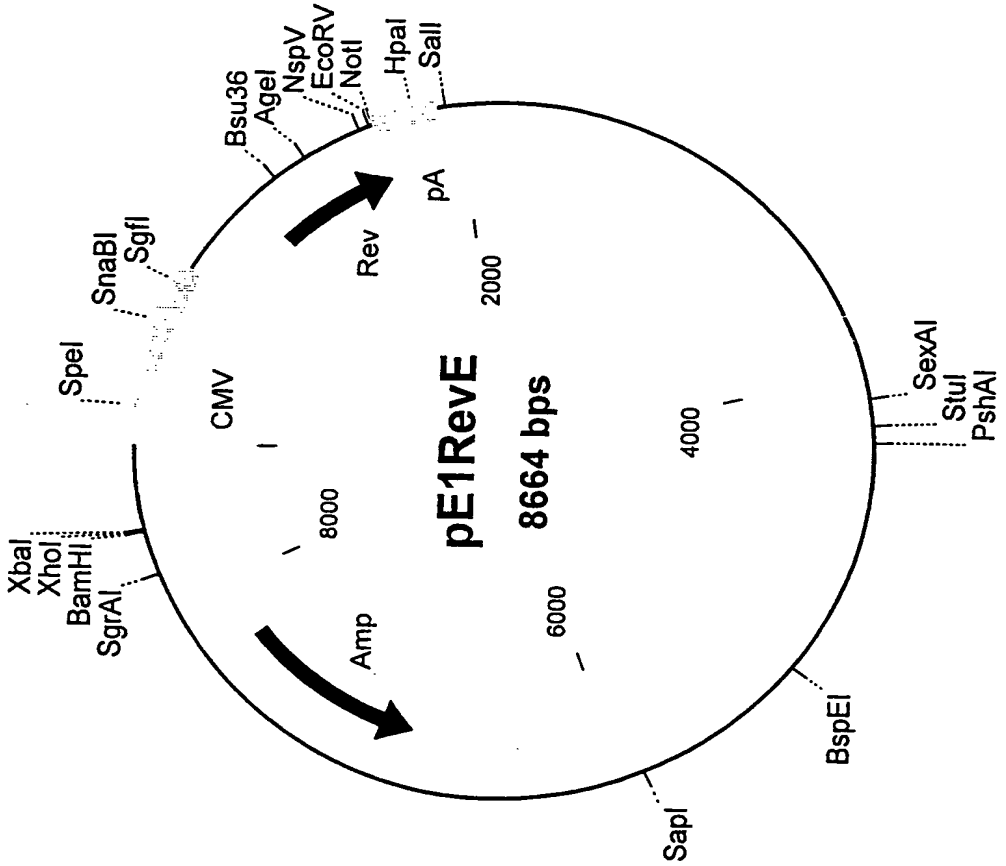


Figure 21

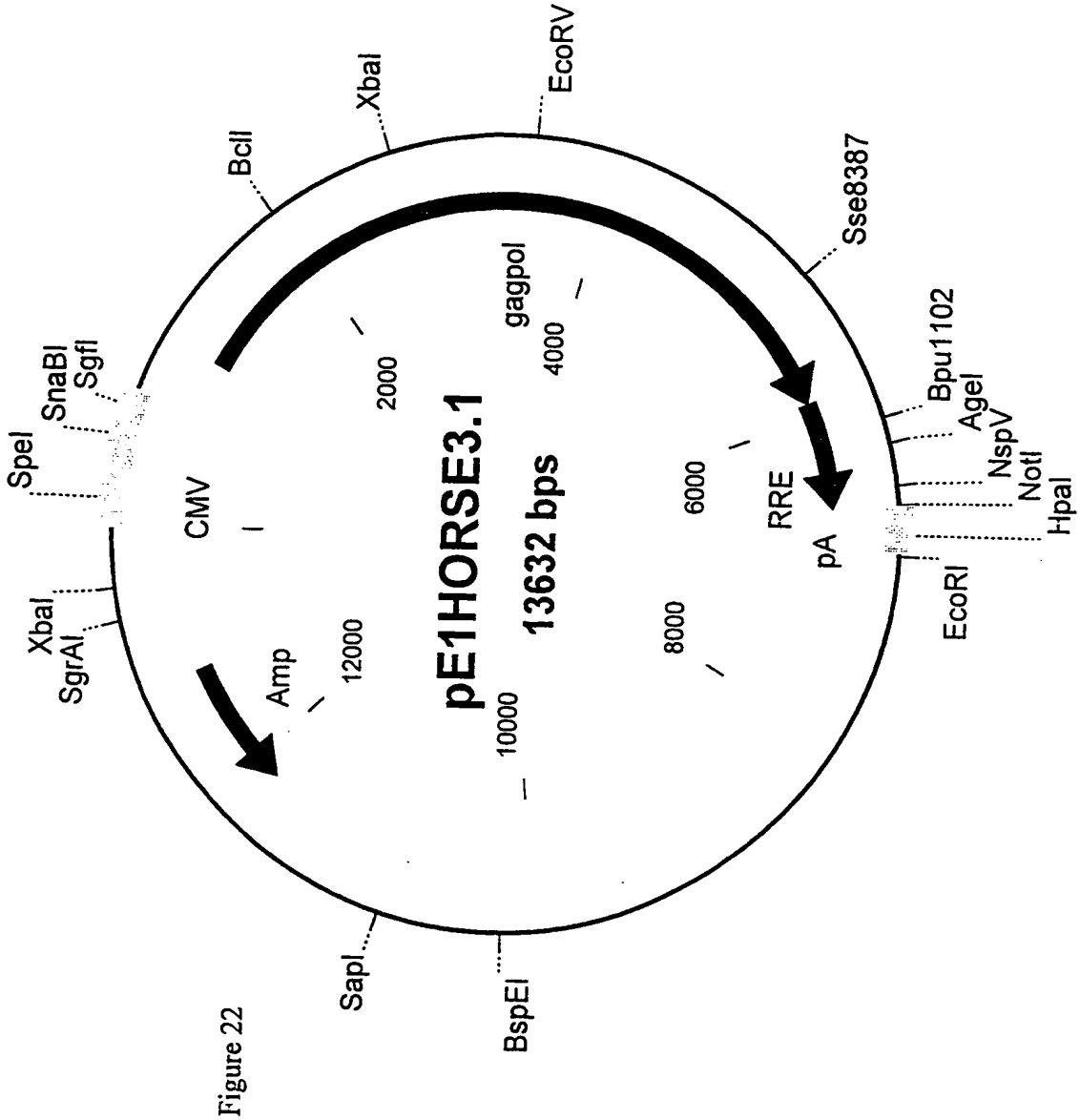

pE1HORSE3.1
13632 bps
2000
4000
6000
8000
10000
12000
gagpol
RRE
pA
CMV
Amp
EcoRV
XbaI
BclI
Sse8387
Bpu1102
AgeI
NspV
NotI
HpaI
EcoRI
SpeI
SnaBI
SgfI
XbaI
SgrAI
SapI
BspEI

Figure 22

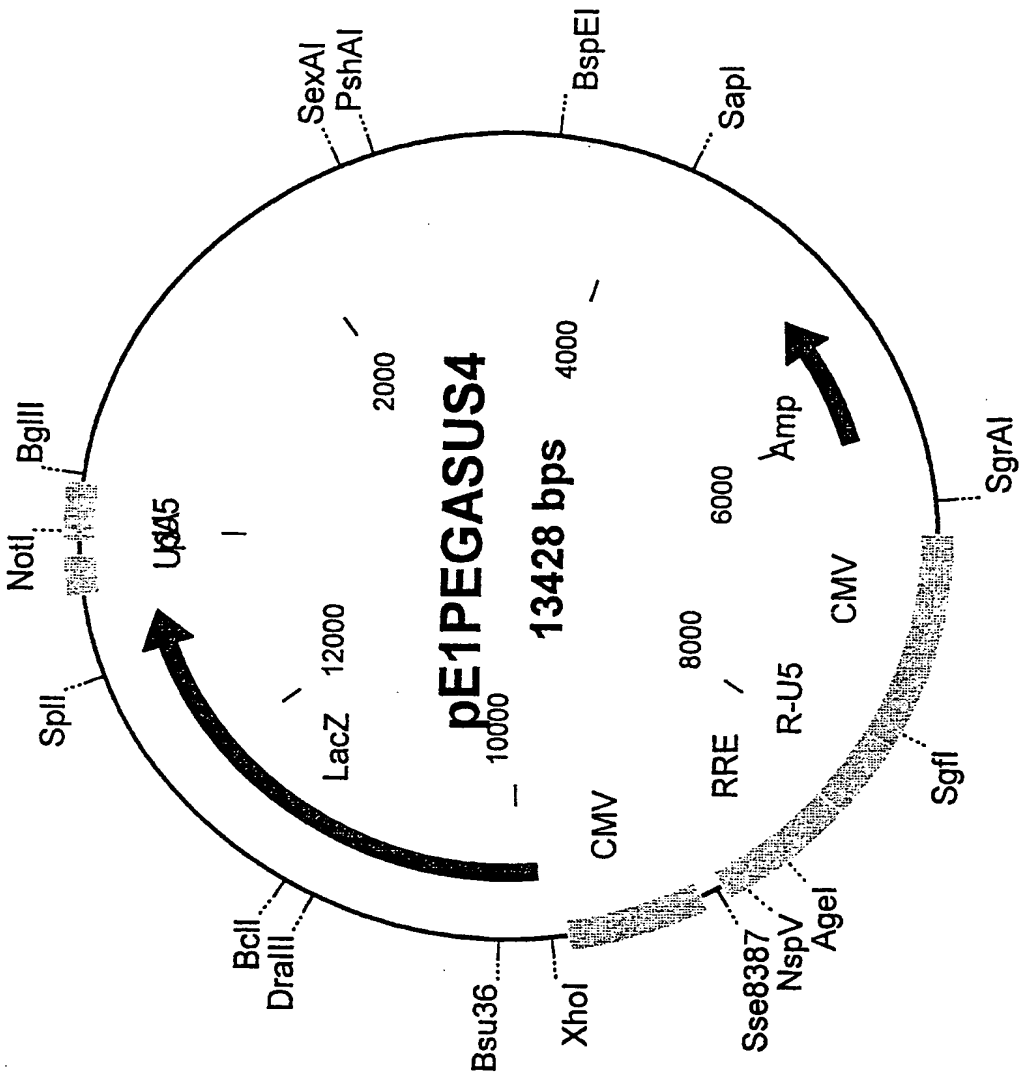


Figure 23

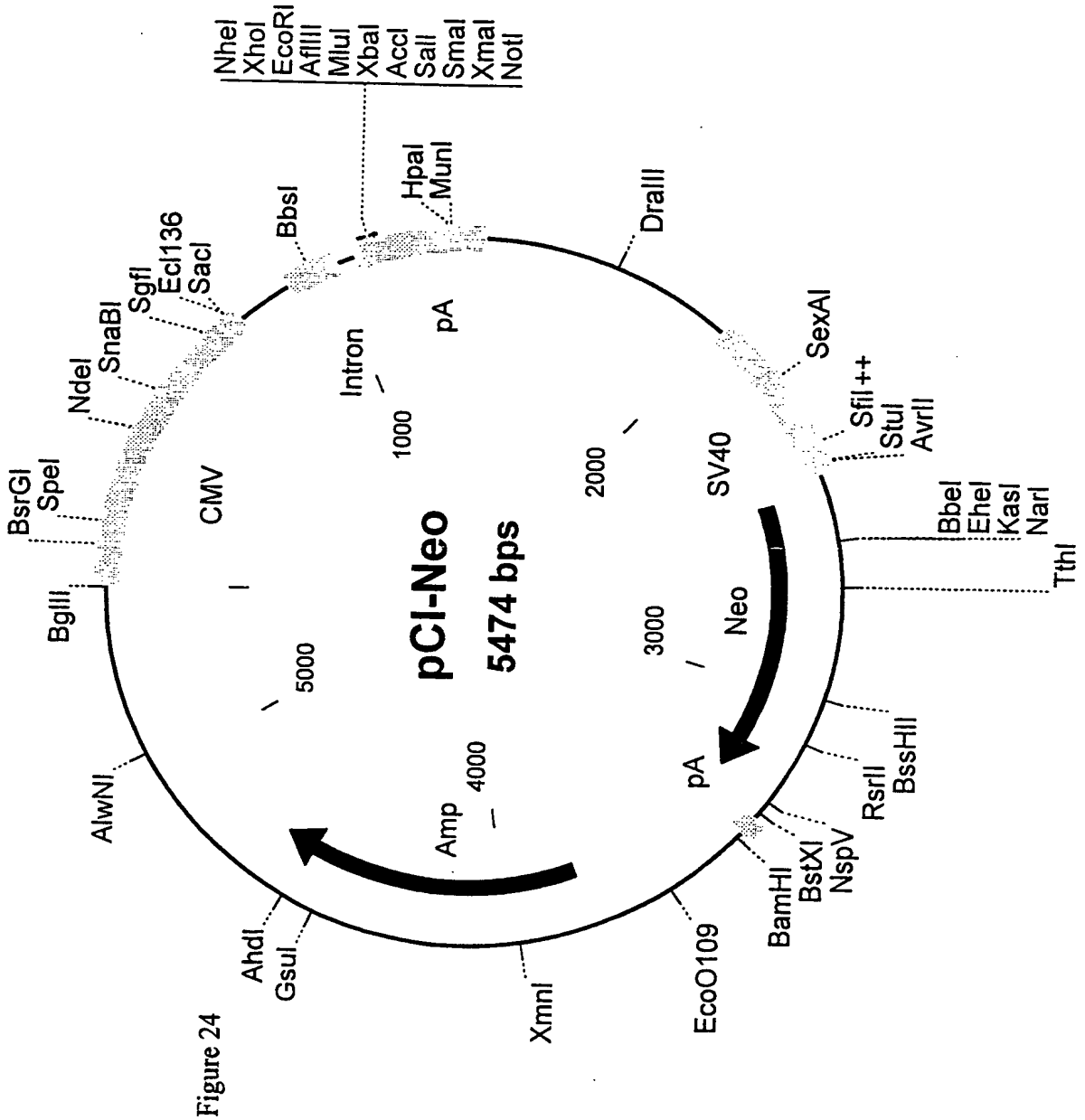

pCI-Neo
5474 bps
CMV
Intron
pA
SV40
Neo
pA
Amp
1000
2000
3000
4000
5000
NheI
XhoI
EcoRI
AflIII
MluI
XbaI
AccI
SalI
SmaI
XmaI
NotI
HpaI
MunI
BbsI
Ecl136
SacI
SgfI
SnaBI
NdeI
BsrGI
SpeI
BglII
AlwNI
AhdI
GsuI
XmnI
EcoO109
BamHI
BstXI
NspV
RsrII
BssHII
DraIII
SexAI
SfiI++
StuI
AvrII
BbeI
EheI
KasI
NarI
TthI

Figure 24

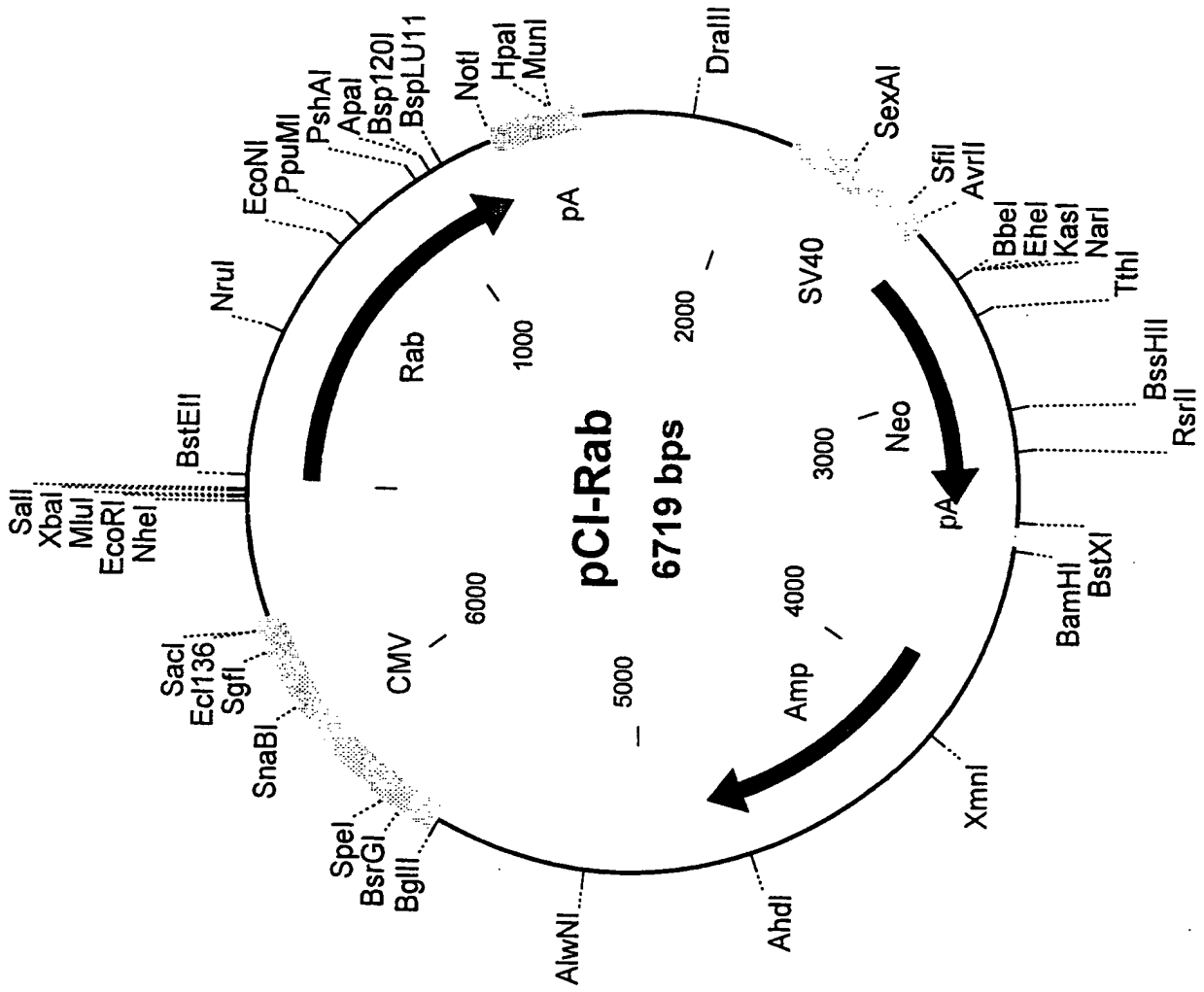

pCI-Rab
6719 bps
1000
2000
3000
4000
5000
6000
Rab
pA
SV40
Neo
pA
Amp
CMV
DraIII
SexAI
SfiI
AvrII
BbeI
EheI
KasI
NarI
TthI
BssHII
RsrII
BamHI
BstXI
XmnI
AhdI
AlwNI
BglII
BsrGI
SpeI
SnaBI
SgfI
Ecl136II
SacI
BstEII
NheI
EcoRI
MluI
XbaI
SalI
NruI
EcoNI
PpuMI
PshAI
ApaI
Bsp120I
BspLU11
NotI
HpaI
MunI

Figure 25

09/937295
PCT/GB00/01091

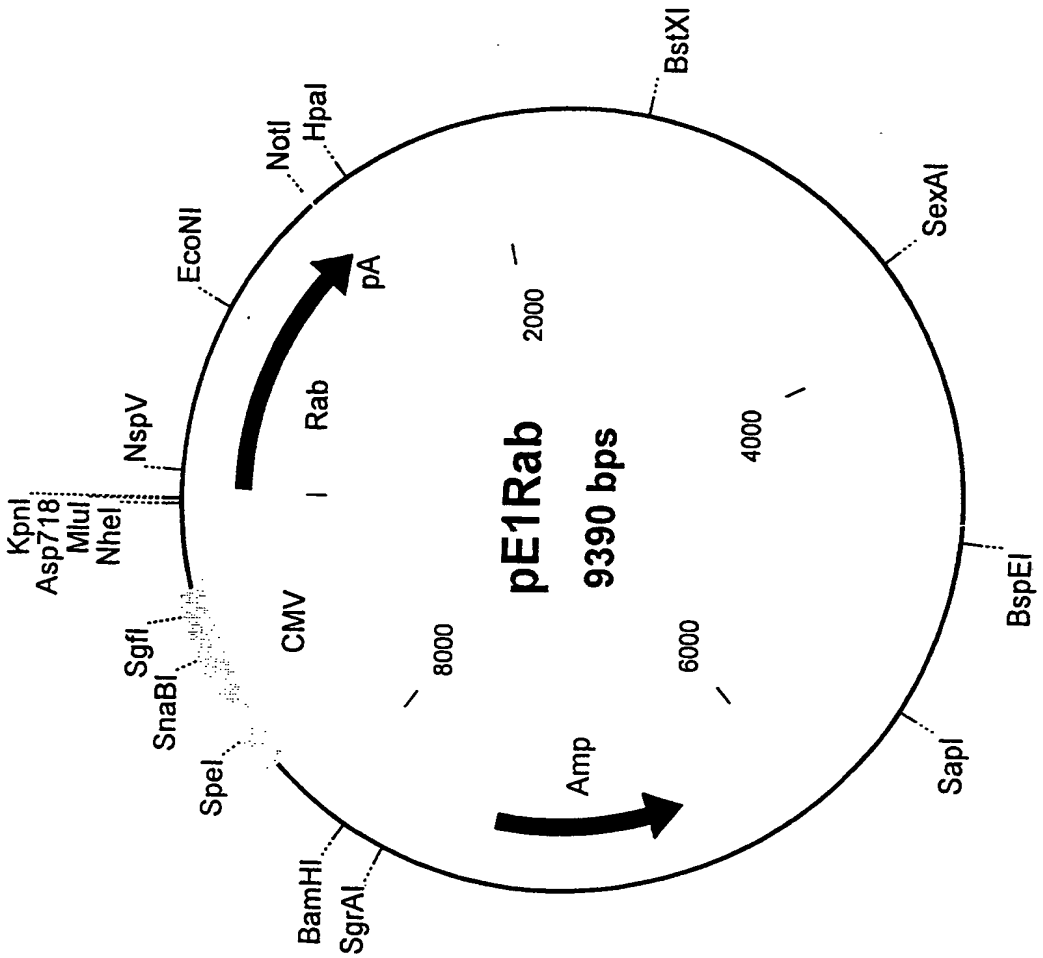
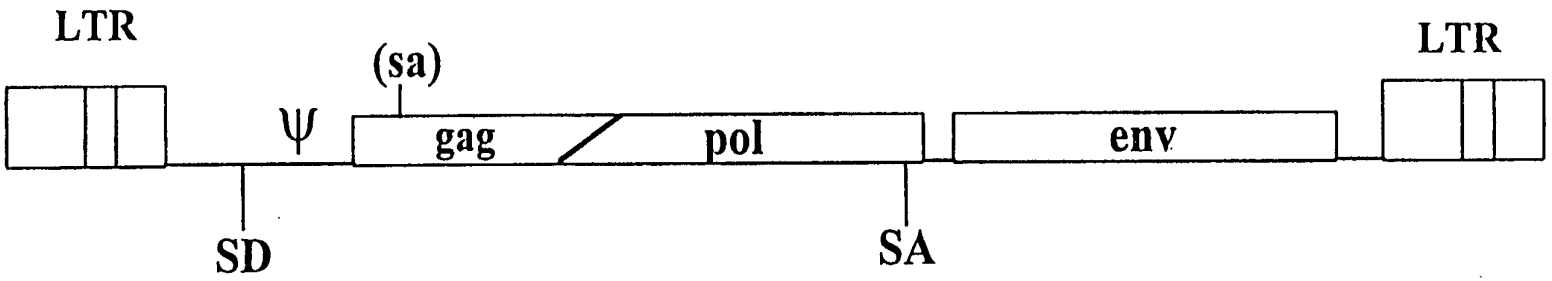


Figure 26

Figure 27a

A) **Natural splicing configuration**

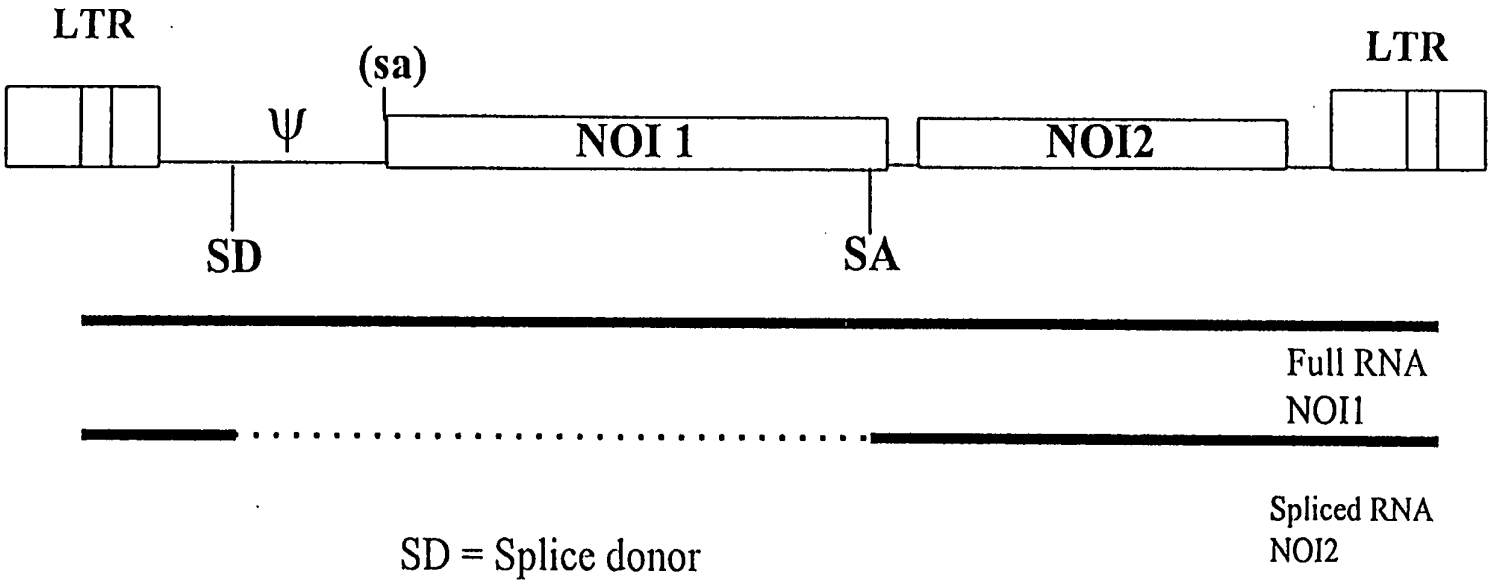


SD = Splice donor
SA = Splice acceptor
(sa) = cryptic splice acceptor
ψ = packaging site

Figure 27b

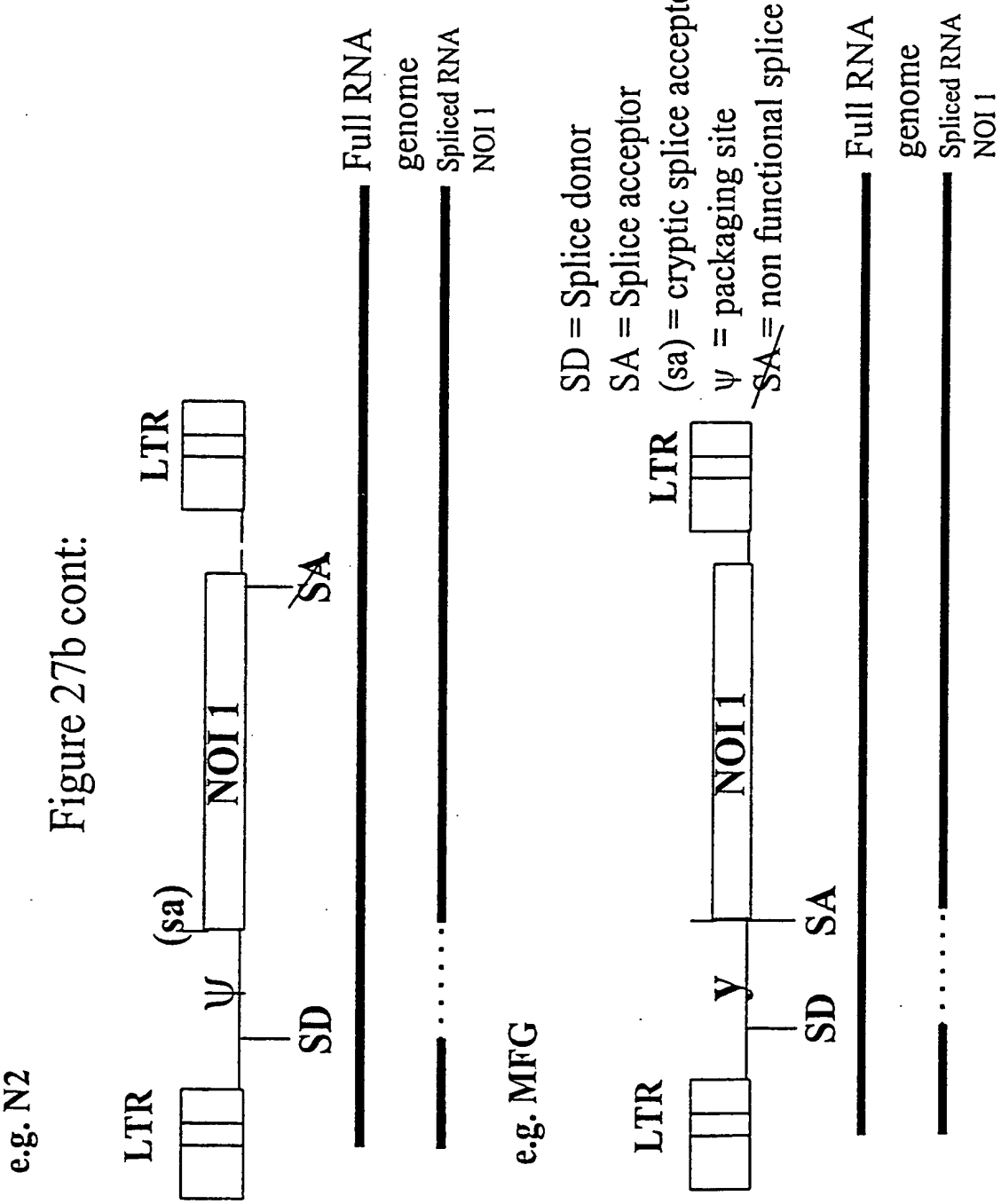
Splicing configurations in known vectors

e.g. LTRSVX



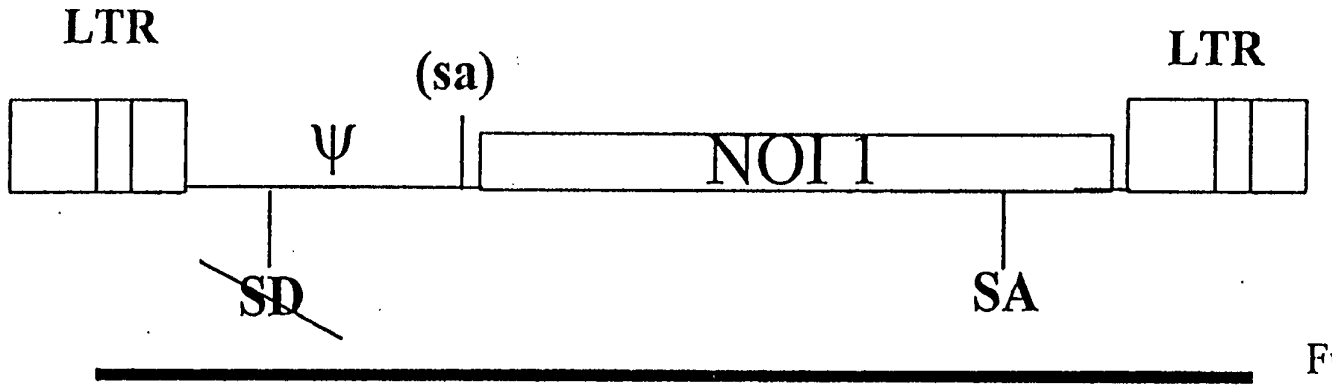
SD = Splice donor
SA = Splice acceptor
(sa) = Cryptic splice acceptor
ψ = packaging site

Figure 27b cont:



e.g pBABE

Figure 27b cont



~~SD~~ = Non functional splice donor
SA = Splice acceptor
(sa) = cryptic splice acceptor
Ψ = packaging site

Figure 27c

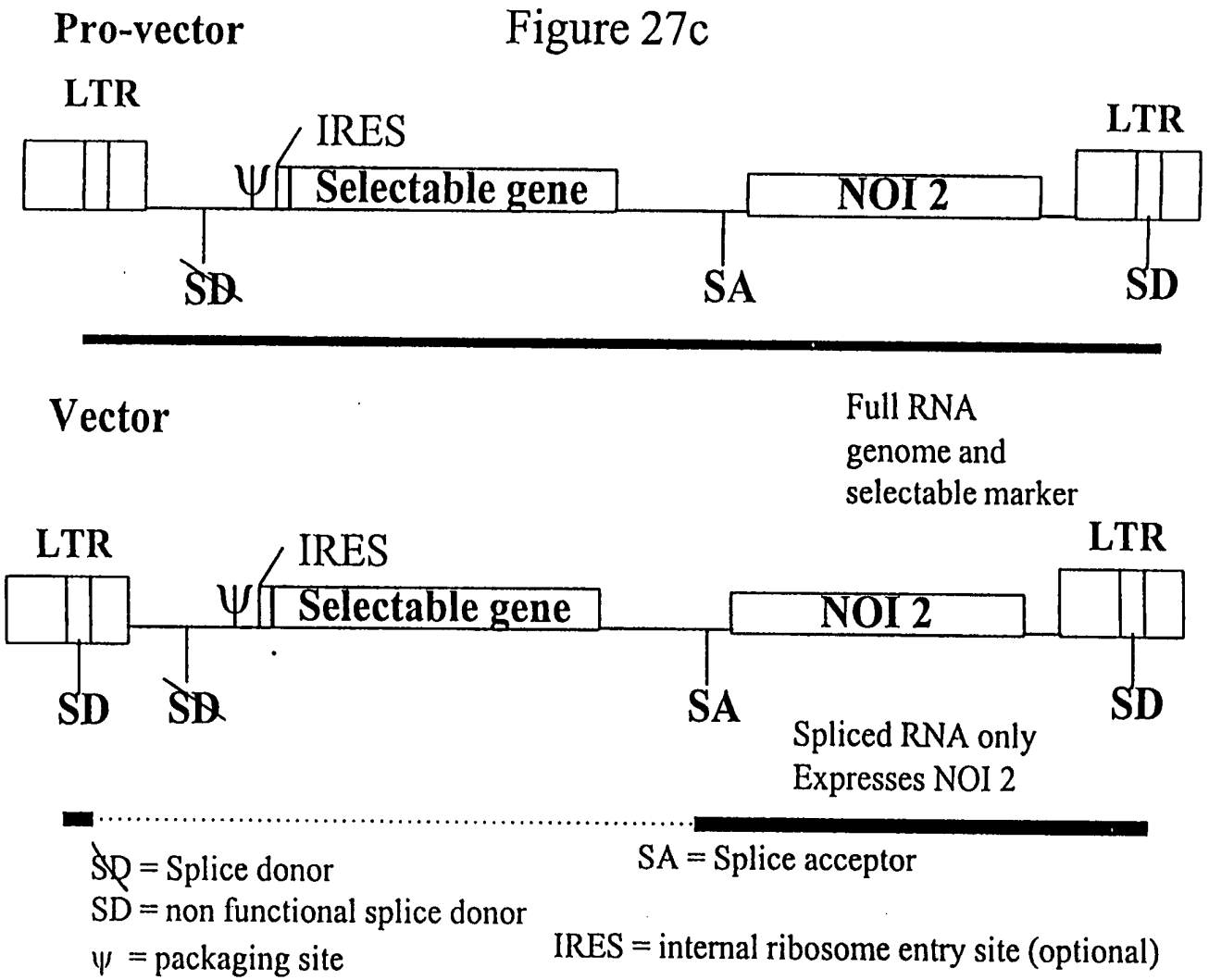
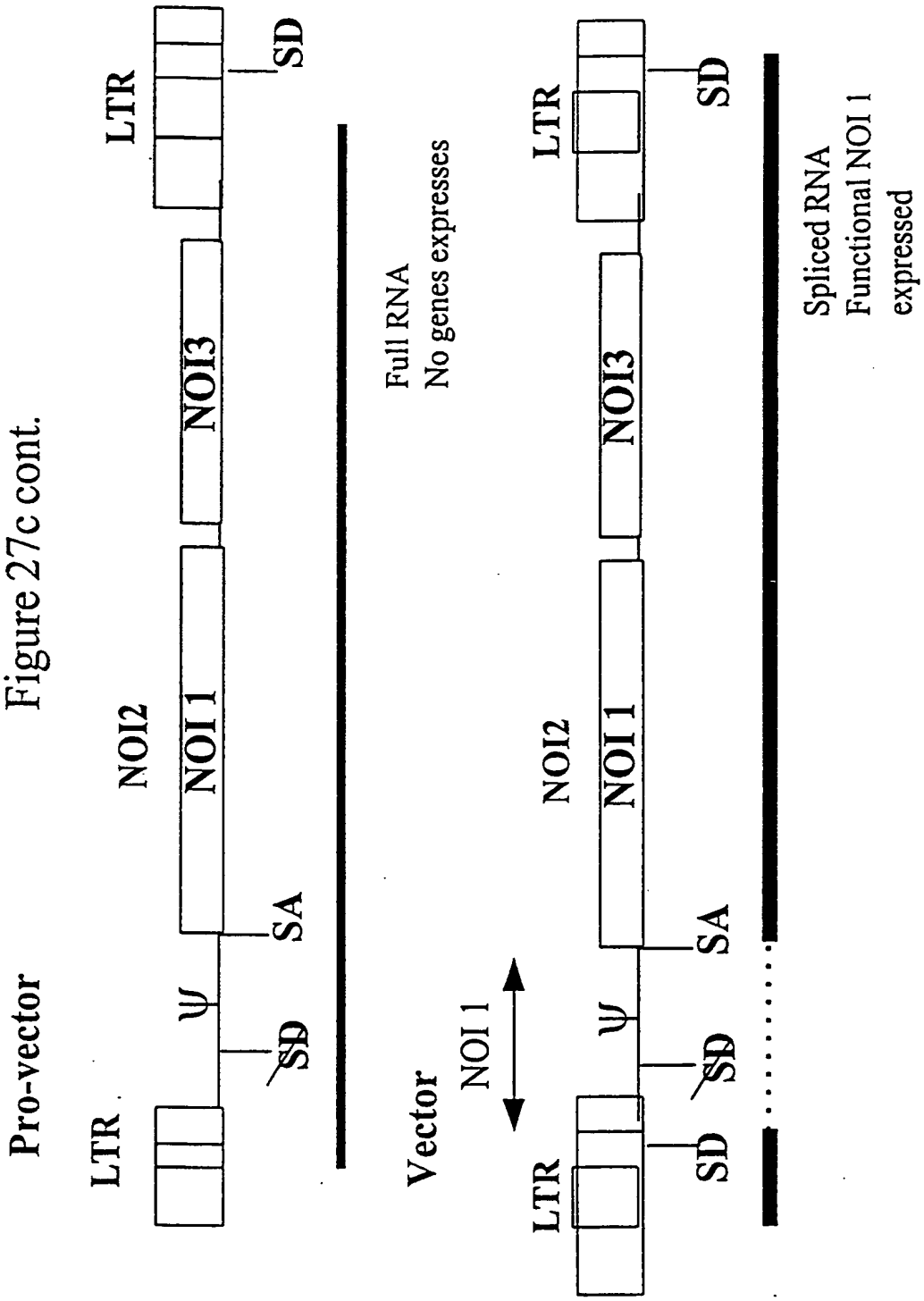

Pro-vector
LTR
IRES
Selectable gene
NOI 2
LTR
SD
SA
SD
Vector
Full RNA genome and selectable marker
LTR
IRES
Selectable gene
NOI 2
LTR
SD
SD
SA
SD
Spliced RNA only Expresses NOI 2
SD = Splice donor
SD = non functional splice donor
ψ = packaging site
SA = Splice acceptor
IRES = internal ribosome entry site (optional)

Figure 27c cont.



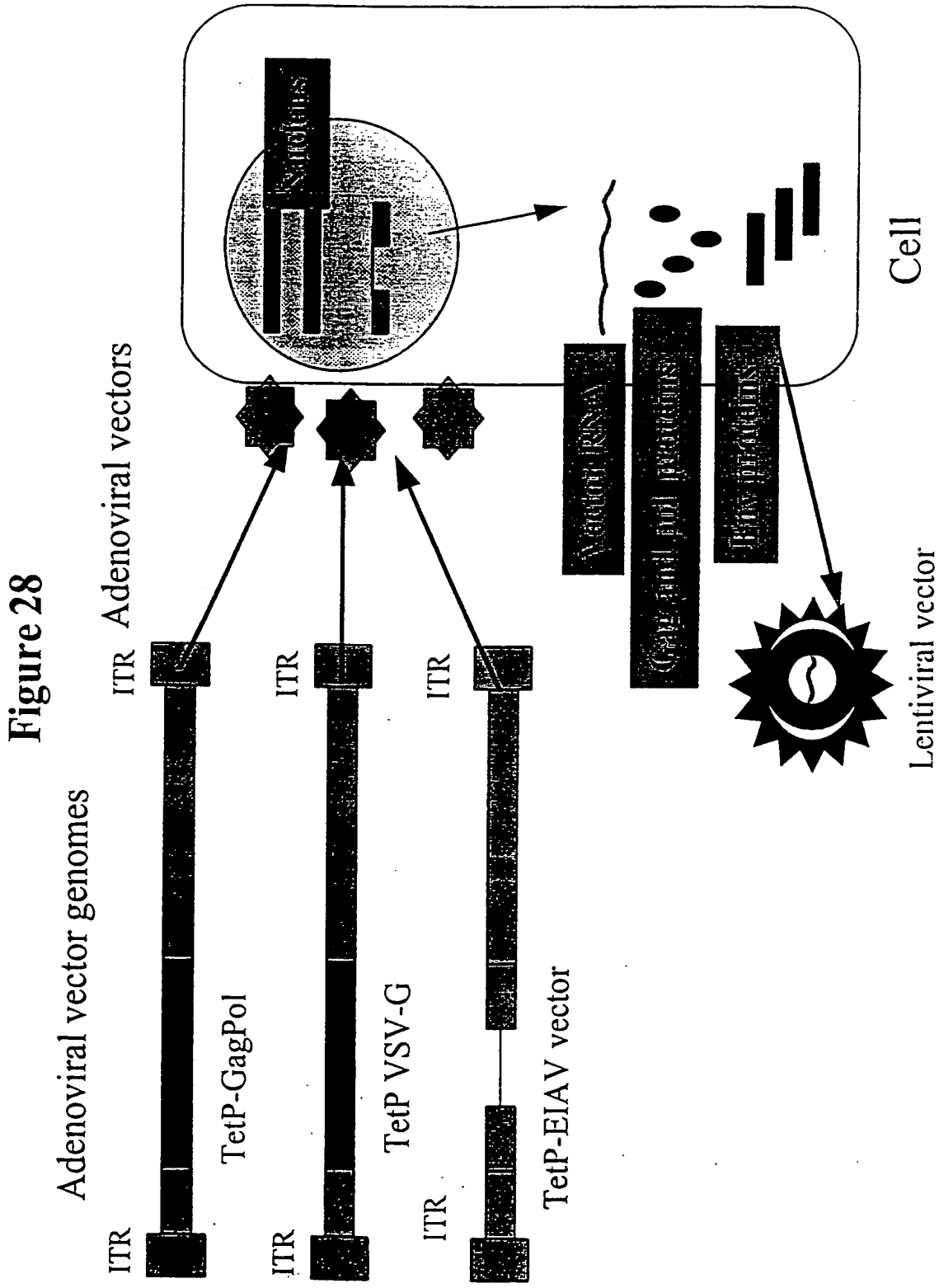

Adenoviral vector genomes
ITR
TetP-GagPol
ITR
ITR
TetP VSV-G
ITR
ITR
TetP-EIAV vector
ITR
Adenoviral vectors
Cell
Lentiviral vector

Figure 28

Figure 29

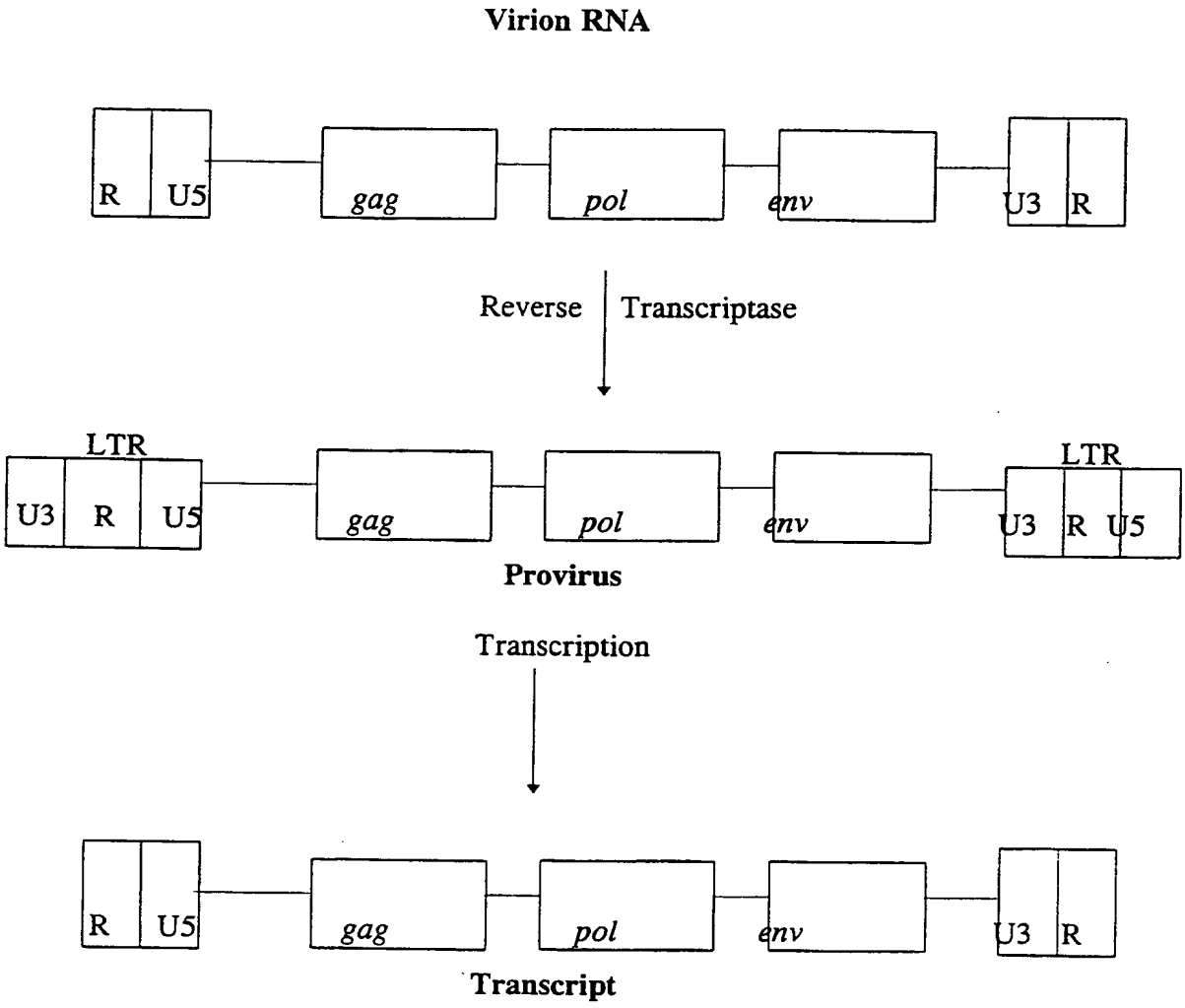

Virion RNA
R
U5
gag
pol
env
U3
R
Reverse
Transcriptase
LTR
U3
R
U5
gag
pol
env
LTR
U3
R
U5
Provirus
Transcription
R
U5
gag
pol
env
U3
R
Transcript

Figure 30

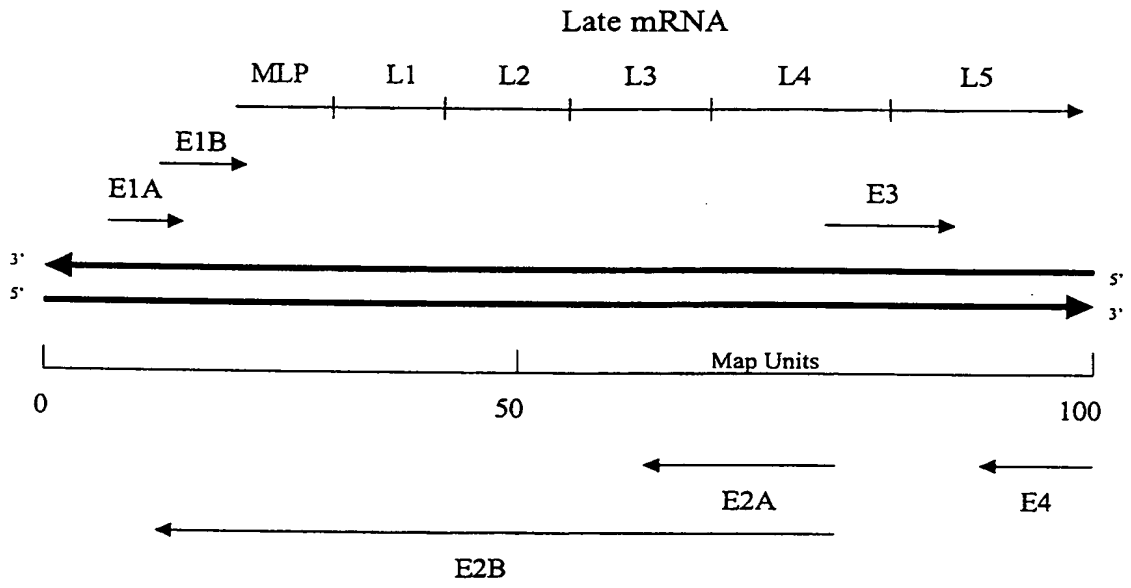
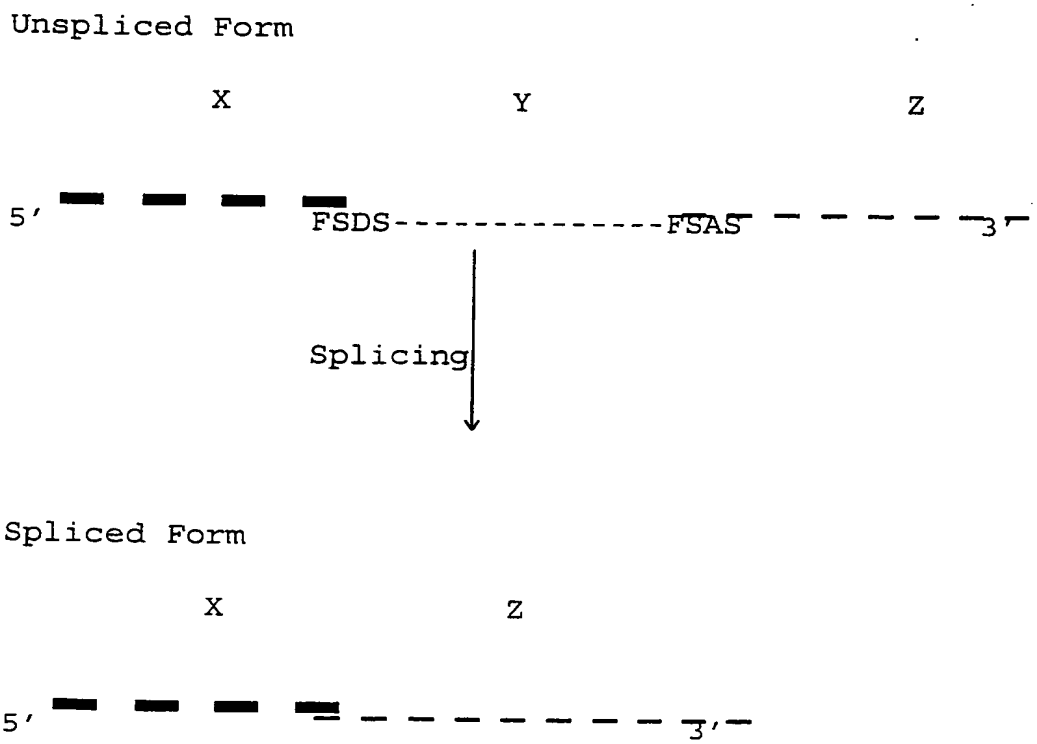

Late mRNA
MLP
L1
L2
L3
L4
L5
E1B
E1A
E3
3'
5'
5'
3'
Map Units
0
50
100
E2A
E4
E2B

Figure 31



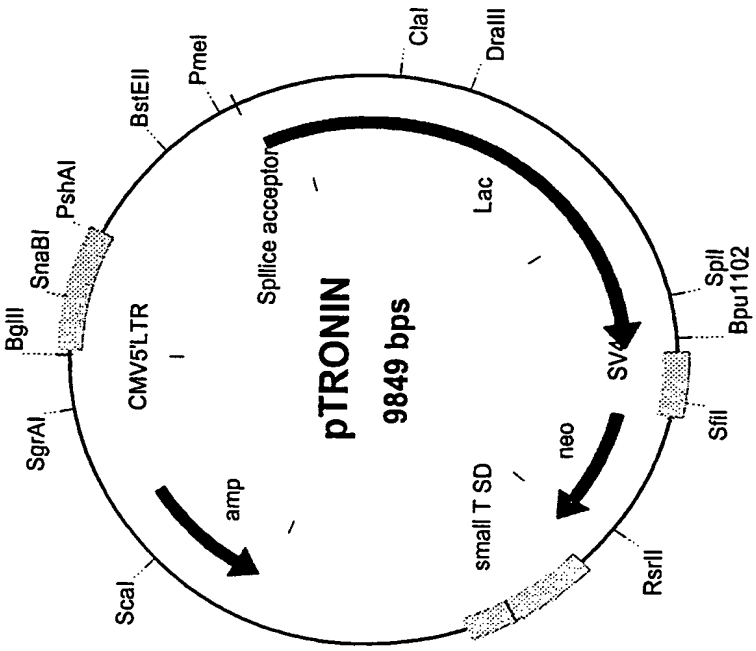
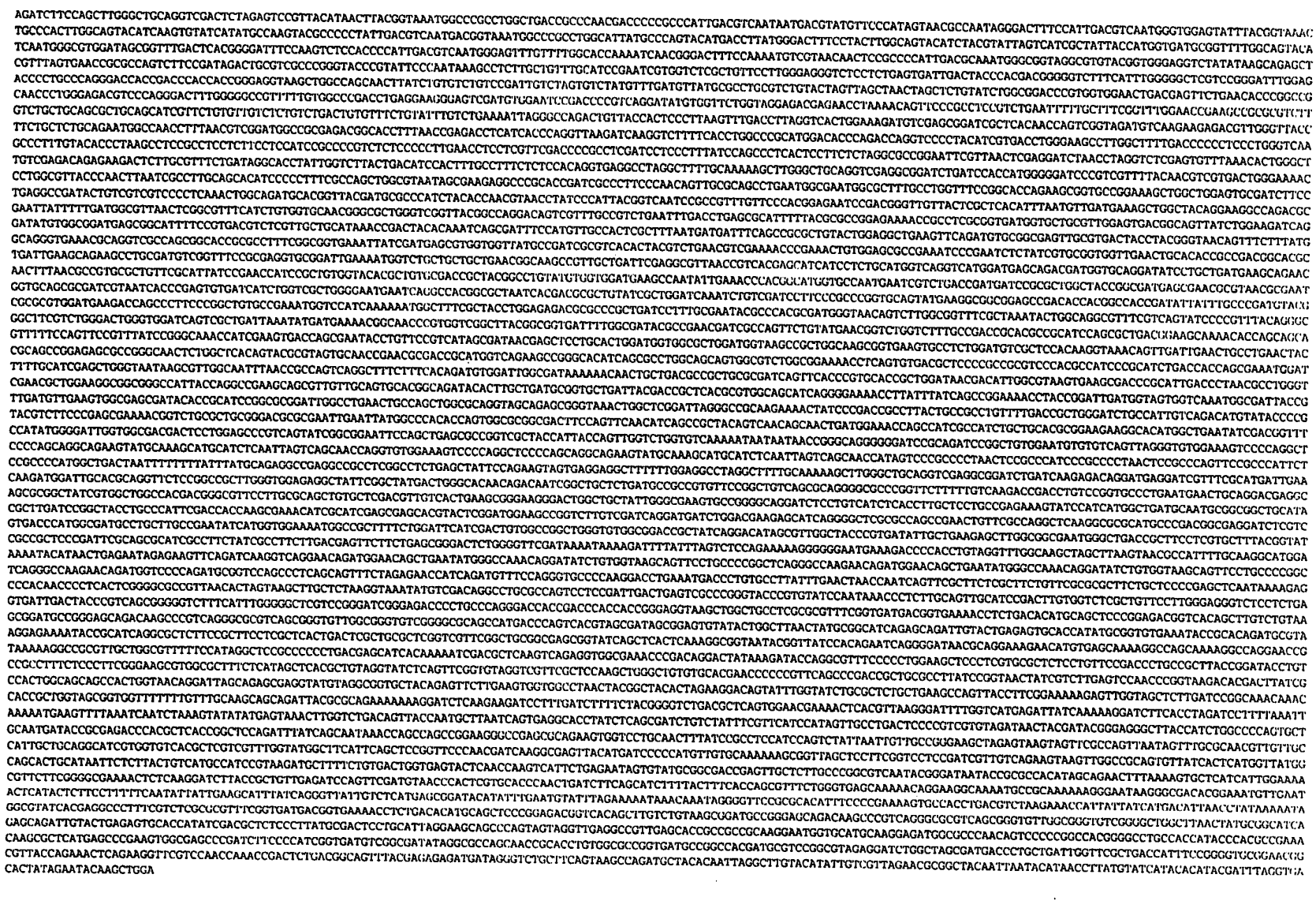

pTRONIN
9849 bps
CMV5'LTR
Splice acceptor
Lac
SV4
neo
small T SD
amp
ScaI
SgrAI
BglII
SnaBI
PshAI
BstEII
PmeI
ClaI
DraIII
SpiI
Bpu1102
SfiI
RsrII

FIGURE 32

FIGURE 33
pTRONIN SEQUENCE



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09/937295

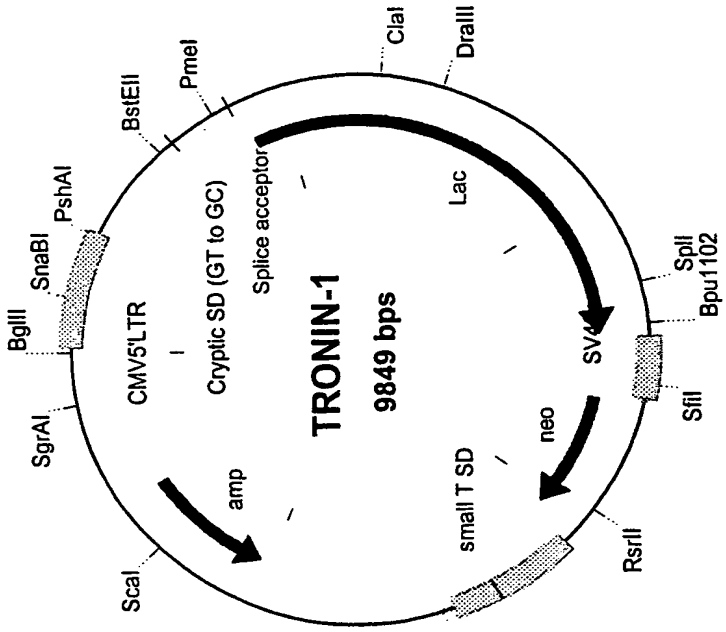
TRONIN-1
9849 bps
ClaI
DraIII
PmeI
BstEII
PshAI
SnaBI
BglII
SgrAI
ScaI
CMV5'LTR
Cryptic SD (GT to GC)
Splice acceptor
Lac
SpII
Bpu1102
SfiI
RsrII
neo
small T SD
amp

FIGURE 34

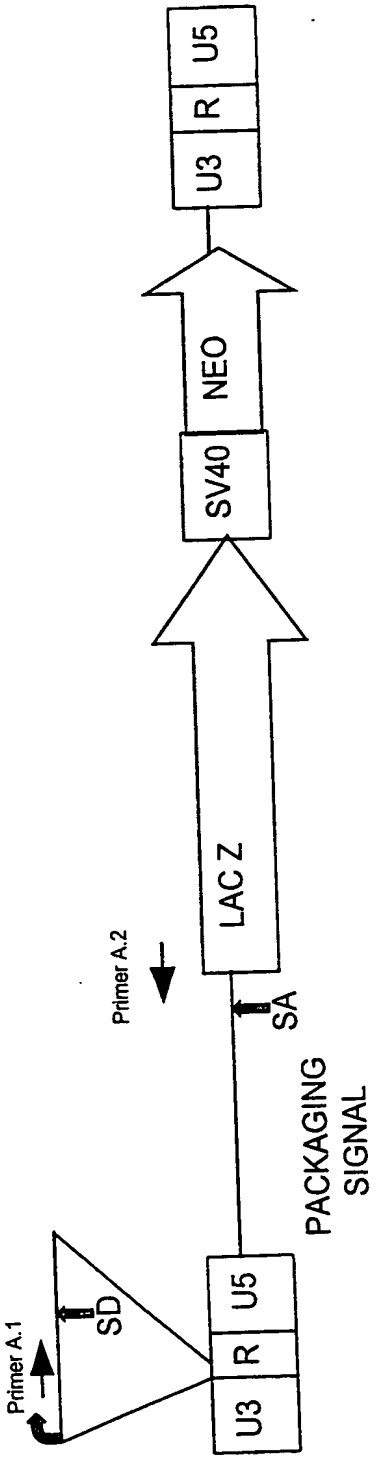
U5
R
U3
NEO
SV40
LAC Z
Primer A.2
SA
PACKAGING SIGNAL
U5
R
U3
SD
Primer A.1

FIGURE 35

FIGURE 36
pTRONIN-1